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Manual of Analytical Quality Control for Pesticides and Related Compounds in Human and Environmental Samples A Compendium of Systematic Procedures Designed to Assist in the Prevention and Control of Analytical Problems

Lafayette Coll, Easton, PA

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Health Effects Research Lab, Research Triangle Park, NC

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MANUAL OF ANALYTICAL QUALITY CONTROL FOR PESTICIDES AND RELATED COMPOUNDS

IN HUMAN AND ENVIRONMENTAL SAMPLES

A Compendium of Systematic Procedures Designed
To Assist in the Prevention and Control of
Analytical Problems

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This manual provides the pesticide chemist with a systematic protocol for the quality control of analytical procedures and the problems that arise in the analysis of human or environmental media. It also serves as a guide to the latest and most reliable methodology available for the analysis of pesticide residues in these and other sample matrices. The sections dealing with inter- and intra-laboratory quality control, the evaluation and standardization of materials used, and the operation of the gas chromatograph are intended to highlight and provide advice in dealing with many problems which constantly plague the pesticide analytical chemist. Many aspects of the problem areas involved in extraction and isolation techniques for pesticides in various types of samples are discussed. Techniques for confirming the presence or absence of pesticides in sample materials are treated at some length. This highly important area provides validation of data obtained by the more routine analytical procedures. The gas chromatograph, being the principal instrument currently used in pesticide analysis, often requires simple servicing or troubleshooting. A section addressing some of these problems is included. Last, but by no means least in importance, is a short dissertation of the value and need for systematic training programs for pesticide chemists.

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FOREWORD

The many benefits fo our modern developing, inducstrial society are accompanied by certain hazrds. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which natianal ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This manual provides the pesticide chemist with a systematic protocol for the quality control of analytical procedures and the problems that arise in the analysis of human or environmental media.

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Director
Health Effects Research Laboratory

ABSTRACT

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PREFACE

The analysis for multi-pesticide residues in biological media surely ranks high among the more difficult and exacting areas of analytical chemistry. The methods are generally complex, involving (a) a series of steps to extract or "strip" the pesticide residue from the substrate, (b) a "cleanup" or partitioning procedure to eliminate a significant portion of contaminants as well as delegating certain pesticidal compounds to certain elution fractions, (c) assay of the residue content via some instrumental technique such as gas chromatography, spectrophotometry, or spectrofluorimetry, and (d) interpretation of the analytical data.

Not unexpectedly, the complexity of a typical residue analysis poses a number of pitfalls into which the analyst may unknowingly stray unless extreme care is constantly exercised to avoid the problems. The purpose of this Manual is to present the field of residue analysis as it is practiced today and to provide guidelines to the analyst to assist him in the implementation of a systematic program of control. Such a program, which will help ensure that the final analytical data are truly valid and representative of the residue profile of the media analyzed, is known as Analytical Quality Control.

Nearly all analytical chemists apply various elements of quality control whether or not it is recognized by this name. Any step taken to produce valid results classifies as quality control. Unfortunately, relatively few analytical chemists follow a program of systematic guidelines and for a variety of reasons. One of the more prominent reasons relates to the time required, and the fact that time is equated with cost and work output. The comment is often heard to the effect that "we practice quality control when we are not busy".

Analytical data are always produced for some kind of decision-making, whether it be to determine that some agricultural commodity is in compliance with established tolerances (regulatory), or to establish the profile of pesticide pollution in a waterway (monitoring). Closely controlled data provide management with reliable evidence suitable for use in a court or sufficiently reliable to make accurate assessments of the degree of pollution in some sector of the environment. Uncontrolled or improperly controlled data, no matter how high the volume, are worse than no data at all because of the misleading effects.

Not only will this Manual discuss the problems and precautions in residue analysis so as to serve as a guide in obtaining valid analytical results, but it will describe a quality control approach for monitoring the performance of chemists and laboratories performing residue analyses. The emphasis is on methods for analyzing residues in biological and environmental samples such as tissue, blood, urine, feces, air, water, and soil. Less attention is given to analysis of agricultural and food products, but approaches to quality control and analytical methods are, for the most part, very similar.

PREFACE TO THE FIRST REVISION

This first revision (1978) of the Quality Control Manual has resulted in significant changes in all chapters. As stated in the preface to the first edition of the Manual (above), the purpose continues to be the presentation of guidelines for a quality control program in pesticide residue analysis as well as discussion of the latest pesticide analytical methods, with emphasis on biological and environmental samples. The greatest changes have been made in the following topics. Recognizing the increasing importance of high performance liquid column chromatoggraphy, considerable new material on quality control, principles, and methodology of this procedure has been added to Chapters 3 and 5. The material in Chapter 5 on GC detectors has been extensively revised to reflect current useage patterns and the availability of new commercial detector models. The new technique high performance thin layer chromatography is introduced and the coverage of quantitation by thin layer densitometry is greatly increased in Chapter 6. The latest analytical procedures for pesticide multiresidues and residues of important specific compounds such as Kepone, PCBs, PBBs, dioxins, and ETU are outlined in Section 7. Material on derivatization reactions and GC-MS for confirmation of pesticide residues has been expanded in Chapter 8. Literature references have been increased and updated in all chapters and most sections.

The author and editors believe that the current revision of the Quality Control Manual is an invaluable and indispensible source of information on proper laboratory techniques and the best available analytical methodology for pesticide analysis. Any comments or suggestions for improvements and additions in future revisions will be gratefully received from users of this Manual.

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Section 1

GENERAL DESCRIPTION OF PESTICIDE RESIDUE ANALYTICAL METHODS

A pesticide residue analysis usually consists of five steps:

(1) Sampling.

(2) Extraction of the residue from the sample matrix.

(3) Removal of interfering co-extractives ("cleanup").

(4) Identification and estimation of the quantity of residues in the cleaned-up extract, usually at very low levels (e.g., 10^{-9} to 10^{-12} grams for gas chromatography). To obtain this sensitivity, selective determinative methods such as chromatography are usually required.

(5) Confirmation of the presence and identity of the residues.

The exact nature of each of these stages is dictated by the specific pesticide(s) and sample substrate involved. A brief discussion of general aspects of these steps follows:

1A SAMPLING

The aim of sampling is to provide a reproduction of a portion of the environment, on a scale that enables the sample to be handled in the laboratory. Analytical results are meaningful only if collected samples are truly representative and meet the goals of the monitoring study or program. The sites, techniques, and frequency of sampling and the size and number of samples must allow the analytical results to be statistically evaluated and replicated at a later time for confirmation. If storage of samples before analysis is necessary, it must be proven that alteration in the nature or amount of pesticide residues does not occur. Samples may be composited or subsampled prior to analysis.

1B EXTRACTION PROCEDURES

Environmental and biological samples generally cannot be analyzed directly for pesticide residues because the level of the desired residue may be too low and the levels of interfering constituents too high. It is usually necessary to extract the desired constituent(s) from the substrate and to purify and concentrate it (them) prior to determination.

A solvent or mixture of solvents should be used for extraction that is at least 80 percent efficient, selective enough to require a minimum of cleanup, and does not interfere with the final determination. Optimum extraction conditions should be found by recovery studies for each analysis in terms of solvent polarity, and in the time and manner of contact between sample and solvent. Simple washing of the whole sample may be adequate for surface residues of foliage or vegetables and fruits, but Soxhlet extractors, blenders, and tumbling or shaking devices are used for most samples. Hexane or hexane-acetone mixtures are typical solvents for non-polar, fat-soluble organochlorine pesticides and benzene, chloroform, dichloromethane, or acetonitrile for the more polar compounds such as organophosphates and carbamates. Acetonitrile is an excellent general solvent for preliminary extraction of unknown residues of a wide polarity range. The more polar solvents, however, remove greater amounts of co-extractives and may complicate subsequent cleanup steps. Sodium sulfate is sometimes added to help extract the more water-soluble compounds. Exhaustive Soxhlet extraction with an appropriate solvent or mixture of solvents is the most efficient method for many pesticides and sample types and can be used to compare with other proposed procedures. Extraction efficiency can be checked most accurately if the laboratory is equipped to apply or incorporate radioactive-labeled pesticides in the sample substrate.

1C CLEANUP PROCEDURES

The amount of extract purification (cleanup) required prior to the final determination depends upon the selectivity of both the extraction procedure and the determinative method. It is an unusual situation, e.g., with some water samples, when extracts can be directly determined without further treatment. Injection of uncleaned samples into a gas chromatograph can cause extraneous peaks, damage to the peak resolution and efficiency of the column, and loss of detector sensitivity. Impure samples spotted for thin-layer chromatography may result in streaked zones or decreased sensitivity of visualizing reagents, while those injected into a liquid chromatograph can greatly shorten the lifetime of an expensive prepacked column. Extracts containing fatty material are especially troublesome. Depending on the extent and nature of the co-extractives and the pesticide residue, partition between immiscible solvents, liquid chromatography (column or TLC), distillation, sweep co-distillation, selective photodegradation, chemical reactions, and reaction gas chromatography (alkaline pre-column) are most often used for cleanup individually or in various combinations.

For adsorption chromatography, extracts are concentrated to a small volume, applied to the top of a Florisil, charcoal, alumina, silica gel, or mixed-adsorbent column, and the pesticides are eluted in fractions by passage of one or more solvents while the co-extractives remain on the column or are eluted in different fractions. Elution of a residue in a certain fraction (selective adsorption) is useful evidence for confirmation of identity. The capacity of a column for co-extractives and the uniformity of activity (elution pattern) from batch to batch are important characteristics of adsorbents used for cleanup. Methods are available for activation and deactivation of different adsorbents and for checking the activity level achieved.

Solvents used for partition cleanup of hexane extracts include acetonitrile, dimethylformamide, and dimethylsulfoxide. The latter solvent is used in the widely applicable Wood procedure to elute chlorinated pesticides from a column prepared from a fatty sample mixed with Celite.

Of the procedures listed above, solvent partition followed by adsorption chromatography is most often applied. An automated instrument based on gel permeation chromatography has been shown to efficiently separate lipids from chlorinated pesticides and PCB's and to be advantageous in terms of convenience and speed of processing large numbers of samples.

Cleanup procedures should be chosen in terms of practicality, cost, time, and reagent and equipment availability. The methods chosen should be tested to be sure they allow detection and determination of the pesticides of interest at the desired sensitivity level, with recovery of preferably 80-85% or better, and with removal or separation of adequate levels of background interferences.

Concentration of solutions is often required prior to, during, and after cleanup procedures. Great care is necessary when evaporating to low volumes to avoid losses of the pesticide residue, and evaporation to complete dryness is usually inadvisable. Kuderna Danish evaporators and micro Snyder columns, special block heaters, and rotary-vacuum evaporators are recommended for concentrating solutions containing pesticide residues, and keeper solutions may be added to retard the loss of volatile compounds. All steps in the analytical procedure should be checked for residue loss or degradation by carrying out recovery studies on a spiked control (uncontaminated) sample at different fortification levels.

1D FINAL DETERMINATION METHODS

Chromatographic methods are by far the most widely used for determination of pesticide residues, followed by spectrophotometric and biological methods. The latter include bioassay and enzymatic techniques which are simple, since they require no cleanup, but are non-specific. Enzyme inhibition, when used as a detection procedure after thin-layer chromatography, is a sensitive (low ng detection limits) and selective method for certain organophosphorus and carbamate pesticides.

Spectrophotometric methods are generally less sensitive and less selective than gas or thin-layer chromatography and are useful mainly as ancillary techniques to gas chromatography for confirmation of residue identity or for quantitation of individual pesticides. If selectivity and sensitivity are adequate, colorimetric methods can advantageously be adapted to automated processes. Fluorescent pesticides and metabolites may be determined by fluorometry, which is more sensitive than visible, UV, or IR methods. Since relatively few pesticides are naturally fluorescent, fluorometry is selective; however, removal of fluorescent impurities is often necessary, and this can be difficult.

Paper chromatography provided the analyst in the late 1950's with the first multiresidue method for separation and identification of pesticides. It has been largely superseded by gas chromatography as the primary determinative procedure and thin-layer chromatography (TLC) for screening, semiquantitation, and confirmation. Compared to paper chromatography, TLC offers generally increased resolution, shorter development times, and increased sensitivity. Most pesticide analyses have been performed on 0.25 mm layers of alumina or silica gel, but polyamide and cellulose are also used. Organochlorine compounds are detected at 5-500 ng levels by spraying with ethanolic $AgNO_3$ or incorporation of $AgNO_3$ into the layer followed by irradiation with ultraviolet light. Many organophosphorus and carbamate pesticides are detectable at low ng levels by enzyme inhibition techniques or at higher levels by numerous chromogenic reagents. Fungicides are detectable by bioautography. Polar herbicides and heat-labile, poorly-detectable carbamates, which require formation of derivatives prior to gas chromatography, are particularly amenable to analysis by TLC.

Gas chromatography of pesticides is normally carried out on 90 to 200 cm glass columns packed with single and mixed organosilicone and polyester stationary phases ranging from low to high polarity. Among the most used phases are SE-30, QF-1, DC-200, OV-210, OV-17, DEGS, Carbowax 20M, GE-XE60, Versamid 900, and DC-200/QF-1, OV-17/QF-1, SE-30/OV-210 mixtures. Samples should be examined on two or three columns of markedly different polarity before results are considered conclusive. Glass columns are preferred because they minimize decomposition and are easier to pack for optimum efficiency. After packing, columns are conditioned at an elevated temperature to minimize liquid phase bleeding and to obtain reproducible chromatograms. In some cases, large quantities of the pesticides being determined are injected initially to improve the response of these compounds.

Columns loaded with relatively low percentages of liquid phase generally give superior resolution and sensitivity but become contaminated more easily and are more prone to interactions between solutes and the solid support than more heavily coated columns. Certain pesticides, such as DDT and endrin, are subject to degradation in columns under certain conditions, and these conditions should be avoided.

Organochlorine pesticides are usually analyzed with a tritium or ^{63}Ni electron capture detector with DC or pulsed applied voltages. Though this detector is less specific than the other common pesticide detectors, it can detect low picogram amounts of many halogenated compounds. ^{63}Ni detectors are operable at high temperatures (over 300°C) thus reducing possible problems from contaminants condensing in the detector. Tritium detectors are less expensive and contaminated foils can be easily changed or removed for cleaning. Commercial devices are available for linearizing EC response over several decades of concentration, and the pulsed wide-range ^{63}Ni detector has become especially popular because it can be used with automatic injection systems. The microcoulometric detector can be operated in a mode specific for halogenated compounds with a sensitivity of about 10^{-9} grams of halogen. Organophosphorus pesticides are detected selectively at low ng levels by the alkali-metal thermionic detector and the flame photometric detector in the phosphorus mode (526 nm). Sulfur-containing pesticides may be selectively detected by the microcoulometric and flame photometric (394 nm) detectors. Nitrogen-containing pesticides are detected selectively at high pg to low ng levels by the thermionic detector or the Coulson electrolytic conductivity detector in their nitrogen modes. Several highly selective flameless detectors for determining nitrogen-containing compounds, which have become commercially available, appear to have greater specificity and sensitivity. The Coulson detector can also be operated selectively for organochlorine compounds, and the Hall modification of this detector is sensitive to 100-400 pg heptachlor. Labile, polar carbamate pesticides or their hydrolysis products are often derivatized with a halogen-containing reagent and the resulting derivative sensitively detected with the electron capture detector.

Currently of less importance than the above are the other proposed pesticide detectors, including the phosphorus mode of the microcoulometric detector, the sulfur and pyrolytic modes of the conductivity detector, the halogen mode (negative response) of the thermionic detector, the microwave plasma detector, the sulfur-phosphorus emission detector, and the Beilstein flame detector. Selective detectors have the advantages of simplifying cleanup procedures and aiding residue identification.

Samples and standards must be injected into the gas chromatograph using a consistent and reproducible technique. It is advisable that injected volumes of standards and samples be nearly equal and represent 20-80% of the total volume of the syringe used. Syringes must be well cleaned between injections and injection port septa and liners changed regularly. Standards should be injected before and periodically during the analysis of a series of samples to check the established calibration curve. Cleaner samples require fewer standard injections. A pesticide mixture that indicates the overall performance of the GC system should be injected at least once daily.

Modern high performance liquid chromatography (LC) is being used increasingly for the final, room temperature determination of polar, involatile, or heat-labile pesticide residues without derivative formation. Analytical columns are commonly 25 or 30 cm in length and 2-5 mm in internal diameter; they are packed with small particles of a totally porous or pellicular (porous layer) adsorbent (usually silica or alumina), reversed phase partition medium (e.g., silica to which a liquid phase has been chemically bonded), or ion exchange resin. The mobile phase is pumped through the column at pressures up to 6000 psig. Ultraviolet absorption, refractive index, polarography, and fluorescence detectors have been used, the latter in determining non-fluorescent pesticides by fluorogenic labelling utilizing methods similar to those employed earlier for thin-layer fluorodensitometry. The major disadvantage of LC at present is the poor sensitivity (ca. 10^{-6} to 10^{-9} grams) of commercially available detectors; however, it is often possible to improve detectability by injection of large volumes of extract without adverse effect on column performance or linearity of detector response. Other advantages of LC compared to GC are that only minimum sample cleanup is usually required, and a greater number of separations of greater complexity can be accomplished; the mobile phase plays an active role in achieving resolution, and there is a wide range of stationary phases available for use in combination with a great variety of solvent mixtures and gradient elutions.

Quantitation of residues by scanning of thin layer chromatograms with commercial densitometers is widely applied for analysis of nonvolatile or unstable pesticides or where GC or HPLC equipment is not available. Precision, accuracy, and selectivity are often comparable to those techniques, and sensitivity is in the high pg-to-ng range for many analyses in which detection is made with fluorescence, chromogenic, or enzyme-inhibition reagents. For best quantitative results, spots are automatically applied to precoated, hard-surface plates, detection reagents are uniformly applied by dipping rather than spraying, and samples and standards are developed together on each plate.

Other final determinative methods that have been applied to pesticide residues include polarography for compounds containing an oxidizable or reducible group, either naturally or after derivatization, atomic absorption, activation analysis, and radiochemical techniques. The latter are most often used in metabolism studies, for example thin-layer chromatography of pesticides containing a radioactive isotope combined with autoradiography or radio-scanning of the layers.

1E CONFIRMATORY TECHNIQUES

Three truly independent results are considered necessary for positive confirmation of the identity of a residue. Alternative methods that can be combined are TLC and/or paper chromatography with sorbent-solvent systems of different polarity or different visualization reagents, gas chromatography on columns of different polarity, preparation of derivatives to alter structure and volatility and thereby chromatographic properties, extraction p-values, ultraviolet photolysis, selective GC detectors, and mass spectroscopy. Unlike conventional NMR, IR, UV, etc., the latter method has sufficient sensitivity (as low as 10 ng) for general application to residue identification as well as for confirmation of pesticides in the presence of PCB's. Thus, the directly coupled gas chromatograph-mass spectrometer is a powerful tool for positive identification of mixture components at residue levels. The ability of the high resolution mass spectrometer to measure precise ionic masses has allowed individual pesticides with different elemental compositions to be identified in complex mixtures without prior separation in some cases.

The reliable detection and estimation of pesticide residues is one of the most difficult and demanding tasks an analytical chemist can be called upon to perform. Important commercial pesticides include insecticides, fungicides, herbicides, acaricides, and rodenticides. There are many hundreds of these compounds with greatly differing chemical structures and properties (e.g., organohalides, organophosphates, carbamates, anilines, ureas, phenols, triazines, quinones, etc.). Their determination may involve traces of any of these materials alone or in combination in a great variety of matrices, each with its own peculiar problems.

Further complications arise because metabolic degradation of certain pesticides produces compounds that may be more toxic and of different polarity than the parent pesticide. Examples include metabolically derived heptachlor epoxide and dieldrin, from heptachlor and aldrin, respectively, and oxygen analog metabolites of sulfur-containing organophosphorus pesticides. The analyst should be able to determine the identity and quantity of these metabolites and degradation products as well as the residue of the original pesticide, and extraction and cleanup procedures and chromatographic determinative conditions may have to be modified to accommodate these compounds. Multi-component pesticides such as chlordane, toxaphene, and strobane and their metabolites pose difficult confirmation and quantitation problems. Closely related, non-pesticidal compounds with similar analytical behavior such as PCB's or chlorinated naphthalenes may also be present in extracts, and the analyst must be able to isolate, identify, and measure pesticides of interest while simultaneously separating, isolating, and identifying these related compounds, if necessary. Trace contaminants contained in solvents or reagents, or extracted from plastic apparatus, can give rise to GC peaks or TLC spots which may be confused with pesticides. Positive confirmation of some pesticides is especially difficult because of very similar chromatographic properties of compounds, e.g., dieldrin and "photo-dieldrin".

The amount of effort expended and the choice of confirmatory tests are determined by the importance of the sample, resources available, and the amount of residue present. A possible alternative to testing of every residue is confirmation of selected samples at intervals, when the same residues are apparently present in all samples of a group. If sufficient residue is not available in individual members of a group of samples to permit use of a certain test, purified extracts are often pooled for confirmation.

1F AUTOMATION

Automation of pesticide analyses is presently in its early stages. Totally automated procedures have been developed for analyses not requiring column adsorption cleanup and those in which the final determination is by colorimetry or UV absorption. Many of the colorimetric methods involve enzyme inhibition. For samples requiring more extensive cleanup, manual procedures must be carried out before the automatic technique. Several means for automatic transfer of manually prepared samples onto a gas or liquid chromatography column and a system for automatic cleanup of samples by gel permeation chromatography have been designed. Data systems allow storage of large amounts of data with computerized printouts that increase the speed and efficiency of analyses. Although advances in automation are being reported at an ever-increasing rate, available systems are generally useful only for well-defined samples containing known pesticides. A skilled analyst using conventional, non-automated procedures is still required to carry out successfully multiresidue analyses of complex samples containing an unknown variety of pesticides and interferences. A proven, completely automated procedure for multiresidue analysis as it is usually performed (i.e., extraction, partition and adsorption chromatographic cleanup, and gas or liquid chromatography) is not yet available.

* * * * * *

Since this introductory section is intended as a broad overview of modern residue analytical methods and their quality control, no details have been given. Much of the foregoing material will be discussed more completely in later sections, and specific references to relevant sections of the EPA Pesticide Analytical Manual or other sources will be given. A general bibliography of recent books and reviews on pesticide analysis follows.

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Section 2

INTERLABORATORY QUALITY CONTROL

2A QUALITY CONTROL PROGRAM OF THE EPA ENVIRONMENTAL TOXICOLOGY DIVISION (ETD) LABORATORY

Quality control in the context of this Manual connotes procedures taken to assure the accuracy and precision of analytical results. Qualitative and quantitative determinations by residue analysts are utilized for such important tasks as surveillance or monitoring of pesticide levels in some segment of the environment or the food supply, and, if conclusions and subsequent actions are to be valid, it is vital that the analytical data be reliable. The complex nature and pitfalls of the analytical procedures as outlined in Section 1 require a set of built-in controls to prevent or detect incorrect results. This Manual is dedicated to a program of quality control which will significantly minimize the output of unreliable and invalid analytical data.

The Quality Assurance Section of the Chemistry Branch, EPA-ETD Laboratory in Research Triangle Park, N.C., functions as the coordinating unit for a quality control program involving various laboratories in the EPA regions. This program was inaugurated in 1966 by the Technical Services Section of the Perrine Primate Laboratory, Perrine, Florida, before the Laboratory was moved to North Carolina. Originally, the program was limited to Community Pesticide Studies, National Monitoring, and State Services Laboratories operating under contract with the U.S. Department of Health, Education, and Welfare, and more recently with the EPA to conduct chemical monitoring for pesticide residues in man and his environment. Parts such as the interlaboratory check sample program have now been expanded to include other state and private laboratories cooperating with the EPA.

The quality control program can be broadly divided into two classifications, both of which will be discussed in detail in this and the following Sections. The Interlaboratory control program, which was the first one formalized, involves analysis of uniform samples* by a number of participating laboratories in order to assess the continuing capability and relative performance of each. In addition, this program indicates, on a mathematical basis, the degree of confidence that can be placed in the results of sample analyses, and identifies analytical areas needing further attention. The coordinating laboratory receives data from the participating laboratories on a special report form, processes the data, ranks the laboratories in order of relative performance, and distributes the final results. Details of these procedures and typical sample data are given in Subsections 2.D. through 2.H.

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* The terms "check sample" and "blind sample" are used for the samples prepared and distributed by the coordinating laboratory. The former term should not be confused with the other widely used meaning of "check sample" (or control sample), that is a sample substrate known to initially contain no pesticides, and then spiked to evaluate recovery by a certain procedure.

The Intralaboratory control program, which will be treated in detail in Section 3, assists a single laboratory in improving the accuracy and precision of data produced by its personnel by providing systematic guidelines for top quality analytical methodology and techniques. One feature of this program is the continual, periodic analysis of standard reference materials (SPRM's) by each analyst and recording of the results on a graphical quality control chart. This chart, which is a plot of the analytical results vs. their time or sequence, evaluates periodic performance in terms of both precision and accuracy and includes upper and lower control limits to serve as criteria for remedial action or for judging the significance of variations between duplicate samples.

A "Statistical Terms and Calculations" subsection at the end of Section 2 will explain some basic terms, equations, and operations used in the quality control programs for data handling and calculation and statistical evaluation of analytical results.

2B PROGRAM OBJECTIVES

The objectives of the interlaboratory program are:

a. To provide a measure of the precision and accuracy potential of analytical methods run routinely by different laboratories.

b. To measure the precision and accuracy of results between laboratories.

c. To identify weak methodology.

d. To detect training needs.

e. To upgrade the overall quality of laboratory performance.

2C PROGRAM ACTIVITIES

The interlaboratory program includes the following activities:

a. The analysis of interlaboratory check samples by all participants.

b. Operation of a repository to provide to any non-profit laboratory analytical grade pesticide reference standards, over 400 of which are now available. These are listed in an index available from the ETD Laboratory.

c. Providing uniform, standard analytical methods in the form of an analytical manual also available from the ETD Laboratory.

d. Quality control of materials of uniform standard quality such as precoated GC column packings, cleanup adsorbent, etc. These materials are purchased from commercial suppliers under stringent specifications in bulk lots, and distributed in individual units to EPA laboratories and other facilities under formal contract with EPA to conduct pesticide studies.

e. Providing abbreviated, informal, on-the-job training for specific requirements.

f. Assisting with problems relating to analytical methodology by phone, mail, or on-site consultations.

g. Operation of an electronic facility for repair, overhaul, design and calibration of laboratory instruments.

2D TYPES AND PREPARATION OF SAMPLE MEDIA

The check sample program is probably the most important interlaboratory activity since all allied activities closely depend on it. Samples used in the program are mixtures of pesticides in a substrate ranging from pure solvent, in the simplest case, to those media routinely analyzed by the participating laboratories, such as fat, blood serum, gonad, brain, and liver tissue, water, soil, and simulated air samples (ethylene glycol trapping solvent spiked at concentration levels found in actual air samples).

As an example, a description is given of the preparation and handling of a blood interlaboratory check sample by the coordinating laboratory: General population serum samples are obtained from a local blood bank, typically in 300 ml bottles. The frozen samples are thawed in a refrigerator (2-3°C), poured together into a stainless steel container (previously rinsed with acetone) and mixed well. Approximately four liters of serum have been sufficient for the program for one year. Experienced chemists analyze the pooled serum to establish the base level profile and to be sure no gross contamination is present. Part of the sample is then divided into small storage bottles with Teflon-lined caps and stored in a freezer (-18 to -23°C). The remainder is stored in bulk for later spiking.

Sub-samples are mailed to participating laboratories to serve as their interlaboratory check sample and to provide sufficient intralaboratory standard reference material (SPRM) for six months. Each laboratory supervisor requests in advance the amount of sample required for the latter purpose based on his estimated routine sample load (see Subsection 3D). A careful study has indicated no need to mail the samples frozen since neither pesticide nor sample degradation has been observed in a 3-to 4-day period. After removing the amount required for the interlaboratory check sample, personnel at each laboratory sub-divide the remainder into small vials which are stored continuously in a freezer. Individual vials are removed as needed to provide 2.0 ml intralaboratory SPRM samples.

The next time an interlaboratory blood sample is required, the same pooled base sample is spiked with pesticides common to blood. This sample will allow the participating laboratories to test their recoveries at high-pesticide levels, thereby simulating analysis of routine samples from individuals occupationally exposed to high pesticide levels. Again, enough sample will be provided each laboratory to serve both as interlaboratory sample and intralaboratory SPRM's for six months.

The same basic procedure is used for other check sample substrates. Rendered chicken fat from a poultry plant has been used for fat samples, while animal brain, gonad and other tissue check samples have also been prepared. It is anticipated that urine samples for testing certain procedures will be supplied in the future.

With the check sample, each participant receives a covering letter providing the protocol for handling the sample. The time allowed for analyzing and reporting results corresponds to the normal time for processing a similar routine sample.

Since the interlaboratory check sample will be recognized as such by the chemist at the time of analysis, he is likely to give special care and attention to it; also, the best chemist in the laboratory may be assigned the sample in the first place. Therefore, poor results on an interlaboratory check sample must be considered a very serious matter since they will often represent the very best work the laboratory produces.

The importance of the interlaboratory check sample program is indicated by a number of actions that were initiated toward standardization based on information obtained over the years. These include distribution of pretested Florisil cleanup adsorbent and GC column packings and frequently updated standard analytical methods.

2E REPORTING FORMS

Laboratories are requested to report their results on special forms. The forms are designed to provide supplemental operating data in addition to numerical results of the analysis. The standard reporting form, with detailed instructions for completion on the reverse side, is shown as Table 2-1. The data and information supplied by each laboratory include the sample size, extent of concentration of the sample extract, injection volumes, elution cuts if column cleanup is required, all instrumental operating parameters, identity of the GC column, and the numerical

TABLE 2-1

REPORT OF INTERLABORATORY CHECK SAMPLE
Electron Capture Detection

LAB. CODE NO._____ SAMPLE NO.___ COLUMN__________________ COL. TEMP.____°C. COL. LENGTH___FT. DETECT. TEMP.___°C. INLET TEMP___°C

TRANSFER LINE TEMP.______°C. CARRIER FLOW___ml/min. PURGE FLOW____ml/min. CHART SPEED___in/min ORIG. SAMPLE SIZE________

PREVALENT STAND. CURRENT_______%f.s.d. at attenuat. of___x___ ELECTROMETER SS E 2 (Circle)

PESTICIDE COMPOUND	VOL. OF FINAL EXTR. (ml)	INJECT VOLUME (ul)	STANDARD INJECTION			SAMPLE INJECT.		RETENTION REL. TO ALDRIN		(ppb) RESIDUE	CHROMATOGRAM NUMBERS
			PICO-GRAMS	PEAK HT. OR AREA	ATTENU-ATION	PEAK HT. OR AREA	ATTENU-ATION	STD.	SAMPLE		

1. Source of method used (Cite journal, volume, page, year or other source)

2. Briefly state modifications, if any.

3. Method and temperature of extract concentration (circle) Air Blowdown Nitrogen Blowdown
Kuderna-Danish Simple Boiling Roto-Vac Other__________________ Temp.________°C

Refer to back of sheet for instructions for completion of report.

Do you wish your chromatograms returned? YES NO
(Circle)

____
Signature of Chemist Doing Work

INSTRUCTIONS FOR COMPLETION OF REPORT

1. This form is to be used only for determinations by electron capture detection. Results obtained by flame photometric detection to be reported on separate form so designated.

2. Use one report form for each GLC column and show under RESIDUE only those values you want to appear in the final summary of results. For example, if a given compound is quantitated on two columns, report only the value in which you have most confidence.

3. All data requested on the report form are meaningful for a complete evaluation of the analytical performance. Therefore, supply complete information under all columns on the form.

4. In case any compound appears in more than one cleanup elution fraction, report the quantitative value for each fraction separately. We will sum to obtain your final total value.

5. Under the column headed VOL OF FINAL EXTR., ml, the numerical value to be placed here should be the final volume and not necessarily the volume achieved by concentration. For example, in handling a blood sample, an extract concentration down to 1 ml might prove necessary for the quantitation of dieldrin. In this case, the figure 1 would appear in the column opposite dieldrin. However, for the determination of p,p'-DDE, a dilution of the concentrated extract up to 10 ml may be indicated. In this case, the figure 10 would appear opposite p,p'-DDE.

6. Each chromatogram of sample and standard shall be sequentially numbered. These numbers are then to be written on the appropriate lines in the column headed CHROMATOGRAM NUMBER. Two numbers should then appear opposite each pesticide reported, one representing the standard chromatogram, the other representing the sample.

7. Include with the chromatograms a standing current profile for each E.C. detector used. Label each step with the polarizing voltage for that step. Record information as to (1) column used during determination, (2) flow parameters - purge and carrier, (3) temperature of column, (4) attenuation used.

8. Mail only original chromatograms with your report. Fold chromatograms for each column in accordion fashion from one continuous roll. Do not mail photocopies. Chromatograms will be returned if you so indicate in the space provided on the form.

9. With your report, include all chromatograms related to the sample work whether used for quantitation or confirmation. However, for those columns used for confirmation only, include all data on the report form except a final quantitative value.

data and original chromatograms upon which all calculations are based. The chromatograms must be clearly identified so that they may be related to the data on the reporting forms for easy checking of the quantitative results by the coordinating laboratory.

2F EVALUATION OF REPORTED DATA

When the completed reporting forms from the participating laboratories are received in the coordinating laboratory, the quantitative results are entered on a Summary of Results sheet illustrated in the next subsection. If any results appear obviously and grossly erroneous, the laboratory is contacted at once and given a chance to review its work and change the report if a simple computational or transcription error is found. After recording all results, a statistical analysis of the results is made and recorded on the Summary of Results sheet. A relative performance or ranking table is also prepared, establishing a numerical ranking value for each laboratory (Subsection 2H).

After the data evaluations and calculations are made, the completed report forms and chromatograms from the laboratories with the poorer rankings are subjected to detailed examination to determine, if possible, the reasons for the inferior performance. Availability of the actual recorder chromatograms for study is vital because it allows the coordinating laboratory to check such factors as column efficiency, sensitivity of detection, instrumental problems such as baseline noise and improperly adjusted recorder gain, proper operating parameters to produce well-resolved peaks, inaccurate reference standards, and faulty quantitation procedures. A detailed critique is then written, and in cases of extremely poor performance, the laboratory is immediately contacted by phone to apprise it of the poor ranking and to make suggestions to improve its performance.

The reports from all other laboratories are then scanned to locate any irregularities that may lead to future problems. A general letter is drafted, and a copy is mailed to all participating laboratories. The letter discusses common analytical difficulties encountered by several laboratories and offers suggestions that appear to have general applicability for improving compound identification and quantitation. Each laboratory also receives a copy of the Summary of Results, with each laboratory identified by a code number and a copy of the Relative Performance Table. Finally, a private critique of performance is sent to each laboratory exhibiting special need for help (Subsection 2I).

2G SUMMARY OF RESULTS TABLES

Typical summary tables are illustrated as Tables 2-2 through 2-5. Definitions and means of calculating the items included in the statistical report at the bottom of each table are given in Subsection 2K.

TABLE 2-2

INTERLABORATORY CHECK SAMPLE NO. 26, MIXTURE OF STANDAPDS IN SOLVENT

SUMMARY OF RESULTS .

LAB CODE NUMBER	PESTICIDES REPORTED IN PICOGRAMS PER MICROLITER														
	Lindane	Aldrin	Hept. Epoxide	p,p'-DDE	Dieldrin	Endrin	o,p'-DDT	p,p'-DDT	Per-thane	Ethion	o,p'-DDD	p,p'-DDD	Mi-rex	DCPA	β-BHC
FORMULA-TION ⟶	10	10	10	75	20	30	20	100	0	0	0	0	0	0	0
45.	12	46*	53*	379*	145*	87*	32	578*							
47.	10	11	11	88	---	---	---	195*							
48.	9.7	9.2	8.7	73	31	29	24	113							
52.	18*	11	14	29*	16	15	---	---	379						
53.	14	14	14	47	18	87*	---	91							
54.	29*	3.0*	6.0*	---	---	---	---	22*	34			4.0	29	9.0	
66.	9.4	8.0	9.2	66	14	30	14	115							
68.	10.1	10	8.5	76	21.6	---	65*	99							
69.	10.4	3*	---	---	92*	---	24	---							
71.	10	5.0	10	---	100*	60*	---	150*							
72.	13.8	8.8	9.4	91	19.5	42	20	98							
73.	12	12	10	64	19	27	33	94							
83.	11.4	14.1	13.5	59	29	39	32.5	88							
84.	8.5	9.6	8.4	70	10	15	11	94							
85.	8.5	8.3	8.1	75	18	27	20	90							
87.	9.5	10.6	9.6	63	22.8	32	24	99							
88.	4.0*	14	12	78	22	12	28	94							
89.	9.0	8.0	10	70	10	14	23	120							
90.	4.8*	6.9	5.1*	45	11.5	---	41	73							
92.	9.0	10	9.0	90	20	---	46	115							
93.	10	12	81*	22*	32	33	31	125				16			
95.	11	8.0	8.0	80	21	---	---	87							
96.	12.4	12.1	10.9	119*	21.7	35.5	27.6	150*							
97.	9.4	11	9.8	89	23.4	33.6	41	110				13.2			2.5
113.	9.0	8	8.6	69	16.4	29	20.4	94							
113A.	9.2	7.8	9.4	72	16.8	28	20.4	96							
130.	11.2	11.0	11.0	86	23.8	29	26.5	112							
135.	9.8	9.4	9.3	74	14	25	21	104							
137.	11	10	10	73	23	29	27	103							
160.	11	9.0	10	102	---	24	---	97		68					
161.	9.7	9.6	10.4	70	16.9	28	18.5	95							
162.	10.1	12.2	10	77	24.3	36	22.6	101							
163.	14	11	10	78	19	---	36	96				12			
164.	4.5*	4.8	6.3*	18*	13.4	24.9	16.2	56*			20				
Mean	10.5	9.9	10.1	74.0	19.6	27.7	26.2	100							
Std.Dev.	1.56	2.31	1.61	12.9	5.64	7.82	8.54	11.6							
Rel.Std. Dev.,%	14.9	23.4	15.9	17.4	28.8	28.2	32.6	11.0							
Total Error,%	36	4.7	33	36	58	60	116	23							

*Rejected as outliers

TABLE 2-3

INTERLABORATORY CHECK SAMPLE NO. 26, MIXTURE OF STANDARDS IN SOLVENT

SUMMARY OF RESULTS

LABORATORY CODE NO.	PESTICIDES REPORTED IN PICOGRAMS PER MICROLITER								
	Lindane	Aldrin	Hept. Epoxide	p,p'-DDE	Dieldrin	Endrin	o,p'-DDT	p,p'-DDT	p,p'-DDD
Formulation→	10	10	10	75	20	15	20	100	0
1.	9.7	10.3	9.3	71	19.7	14.2	23.7	94	
2.	10	9.9	9.7	75	18.9	13.2	24.7	99	
3.	9.9	10	10	75	18	13	21	83*	12
4.	8.5	11.3	10.4	102*	22.6	15.9	30.4	99	
5.	10.1	9.7	10.4	81	15.3	10.0*	24.2	99	
6.	10.7	9.9	10.2	77	20.2	14.8	27.6	102	
7.	9.5	9.7	9.9	75	21	15.2	21.8	100	
8.	9.8	9.9	8.9	75.5	15.7	15.4	23.8	99	
9.	10	9.0	9.5	72	19.3	17	23	100	
10.	10.2	9.5	9.8	74	20.2	18	26.4	111	
11.	10.3	10.1	10.3	83	18	13	25	100	
12.	10	10.3	10.6	84	20	11.7	23	99	
13.	9.3	8.9	9.2	82	19	14	22	91	
14.[1/]	6.8*	7.2*	7.0*	56*	17.6	13.2	19.5	83*	
1/ Lab recently joined program.									
Mean	9.9	9.9	9.9	77	19	14.5	24	99.4	*Rejected as outliers
Std. Deviation	0.54	0.60	0.52	4.37	1.96	1.78	2.79	4.70	
Rel. Std. Dev. %	5.5	6.1	5.3	5.7	10.3	12.3	11.6	4.7	
Total Error %	12	13	11	14	25	27	48	10	

TABLE 2-4

INTERLABORATORY CHECK SAMPLE NO. 25, SERUM

SUMMARY OF RESULTS

LABORATORY CODE NO.	PESTICIDES REPORTED IN PARTS PER BILLION					
	Dieldrin	o,p'-DDT	p,p'-DDE	p,p'-DDT	β-BHC	HCB
Formulation ⟶	4.0	6.0	45	8.0	0	0
1.	2.2*	5.0	46	8.7		
2.	5.0	5.1	42	9.8		
3.	4.0	5.0	41	10		<1
4.	6.0*	7.9	62*	14*	1.9	
5.	3.7	6.0	39	9.6		
6.	4.2	6.5	53	9.3		
7.	3.5	6.5	50	9.8		
8.	3.9	5.4	46	9.2		
9.	3.8	5.6	44	10		
10.	4.0	4.0	35	8	1.0	
11.	4.1	5.0	44	8.3		
12.	3.9	5.8	39	9.3	1.3	
13.	3.4	4.6	52	7.3		
14.	4.5	5.2	44	8.7		
15.	4.1	4.9	38	9.8		
16.	3.6	5.5	44	9.6		
17.	4.9	5.1	39	8.7		
18.	4.4	8.4	38	10.8		
Mean	4.1	5.6	41	9.2		*Rejected as outliers.
Std. Deviation	0.46	1.11	5.12	0.86		
Rel. Std. Dev. %	11.2	19.7	12.5	9.4		
Total Error %	25	44	24	37		

TABLE 2-5

COLLABORATIRVE SAMPLE NO. 21, SPIKED FAT

SUMMARY OF RESULTS

LABORATORY CODE NO.	PESTICIDES REPORTED IN PARTS PER MILLION							
	Aldrin	Hept. Epoxide	Dieldrin	o,p'-DDT	p,p'-DDD	p,p'-DDE	p,p'-DDT	β-BHC
Formulation→	0.10	0.30	0.70	0.80	0.40	9.0	3.0	0
1.	---	.33	.45	.76	.37	8.70	3.11	
4.	.090	.27	.58	.63	.44	8.66	2.63	
5.	.13	.34	.60	1.02	.47	7.52	2.09	
6.	---	.26	.67	.64	.42	9.32	2.86	
7.	.090	.30	.75	.74	.48	7.5	2.60	
8.	.090	.31	.71	.75	.48	8.80	3.08	
9.	.070	.060*	.54	.67	.44	7.10	2.36	
11.	.060	.27	.050*	.70	.29	7.23	2.90	
14.	---	.32	.52	.80	.53	8.51	2.67	
15.	.10	.30	.80	.68	.37	8.90	3.10	
16.	.080	.31	.70	.73	.40	8.29	3.03	
24.	.060	.020*	.39	.43*	.32	6.70	1.60*	0.020
25.	.090	.31	.69	.72	.48	9.32	2.70	
26.	.080	.22	.63	.53	.33	6.59	3.12	
31.	.050	.25	.33	.50	.24	6.30	2.20	
33.	.060	.25	1.12*	.67	.32	7.56	2.71	
34.	.060	.19	1.12*	.52	.39	9.07	3.53	
Mean	0.080	0.28	0.60	0.68	0.40	8.03	2.73	*Rejected as outliers
Std. Deviation	0.022	0.042	0.14	0.12	0.080	1.00	0.38	
Rel. Std. Dev. %	28	15	23	18	20	12	14	
Total Error %	65	35	54	46	40	33	34	

Table 2-2 shows data from a group of 34 laboratories participating in an interlaboratory control program for the first time as a group entity. The distributed sample consisted of a precise formulation of eight chlorinated pesticide and metabolite standards dissolved in pure solvent in a sealed ampoule; no cleanup steps were required. The mean and standard deviation values were calculated after rejection of the outlying values designated by asterisks. (See Subsection 2Ke for description of fitness test). The precision (relative standard deviation) was considered "good" for this type sample only for the compounds lindane, heptachlor epoxide, and *p,p*'-DDT, "fair" for *p,p*'-DDE, and "poor" for the other four. The overall average RSD for all compounds was 21.6 percent, nearly double the value expected from a group of laboratories with top quality analytical output, such as illustrated by Table 2-3. Total Error values considered "good" include lindane, heptachlor epoxide, and *p,p*'-DDT, aldrin is "fair", and the others "poor". The average Total Error was 52 percent, just outside the "acceptable" level of <50 percent.

Table 2-3 shows, for comparison, results on the same sample (except for a more difficult, lower endrin content) by a group of laboratories that (except for one) had been in the quality control program for several years. The calculated average RSD value is 7.7 percent and the average Total Error 20 percent, both "excellent" performance values. The average total time spent in each laboratory on the sample by this group was 1.5 days. During the earlier years of participation, the data output of these laboratories was similar to that shown in Table 2-2, but continuing participation in both Inter- and Intralaboratory Programs resulted in gradual improvement in performance to the levels shown in Table 2-3. As an example of a factor responsible for the poor results in Table 2-2, the 34 laboratories used 33 different GC columns, while the experienced group represented in Table 2-3 used only the optimum GC columns and the operating parameters recommended in the EPA Pesticide Analytical Manual and in Subsection 5G of this Manual.

Table 2-4 shows typical results for a more difficult blood serum check sample reported by 18 laboratories with experience in the quality control program. The average RSD of 13 percent and Total Error of 33 percent are quite acceptable for this type sample. Table 2-5 shows results from 17 laboratories for a fat check sample. Average RSD and Total Error values are obviously not so good in this case.

2H RELATIVE PERFORMANCE RANKING

A scheme has been developed and tested for the relative ranking of laboratory results in the analysis of multiresidue check samples. There are three essential criteria for a high score in this performance ranking, namely, correct identification of all pesticides present, correct quantitative assay of the pesticides, and non-reporting of pesticides not present. The ranking scheme incorporates all three criteria and provides a numerical score for each.

The maximum possible score is 200 points, 100 for correct identification and 100 for quantitation. A detailed explanation of the calculation procedure follows:

a. Identification

The 100 possible total points divided by the number of compounds actually present yields the point value per compound. Correct identification of all compounds present and reporting of no extra compounds results in a total score of 100 points. A penalty equal to the point value per compound is assessed for each compound reported that is not actually present. For example, if five compounds are present in the check sample, each is worth 20 points. If one is missed and one extra reported, a penalty of 2x20=40 points will be assessed against identification performance. The score in this part would then be 100-40=60 points.

b. Quantitation

The point value per compound is again the total possible points (100) divided by the number of compounds present. Should all reported values coincide exactly with the formulation values (an unlikely situation), the full 100 points are awarded. When a reported value differs from the formulation value, the difference between the two (the absolute error) divided by the standard deviation (previously calculated for each compound) gives a "weighted deviation". This value is subtracted from the point value of the compound to give the quantitative score for that compound:

$$\text{Compound Quantitative Score} = \text{Compound Point Value} - \frac{\text{Absolute Error}}{\text{Standard Deviation}}$$

The total score for this part is the sum of the individual compound quantitative scores.

An important aspect of the quantitative portion of ranking is the role played by the standard deviation for each compound. If the precision of the group for the analysis of a particular pesticide is poor, the standard deviation for that compound will be relatively high. If a laboratory has a large absolute error for this one compound but an otherwise excellent performance, division of the error by the large standard deviation will lower the point loss so that an unduly heavy scoring penalty is not received.

c. Total Score and Sample Results

The total score for laboratory performance is the sum of the identification and quantitation point totals. Table 2-6 illustrates in detail the method of calculation for a hypothetical analysis in which one compound is missed and one extra is reported, resulting in an inferior total score of 125.

Table 2-6

CALCULATION OF TOTAL SCORE FOR RELATIVE PERFORMANCE RANKING

	Formulation pg/µl	Reported Analytical Values pg/µl	Standard Deviation*
β-BHC	30	27	2.10
p,p'-DDE	40	40	1.75
Dieldrin	20	50	2.50
o,p'-DDT	10	Not Reported	0.60
p,p'-DDT	50	47	1.44
α-BHC	None	10	----

*Of all data from participating laboratories

Point value for each compound is 100 ÷ 5 = 20

Identification

β-BHC - 20
p,p'-DDE - 20
Dieldrin - 20
o,p-DDT - 0
p,p'-DDT - 20
sum = 80
-20 Penalty for false identification of α-BHC
60 Total identification points

Quantitation

β-BHC - $20 - \frac{30 - 27}{2.10} = 19$

p,p'-DDE - $20 - \frac{40 - 40}{1.75} = 20$

Dieldrin - $20 - \frac{20 - 50}{2.50} = 8$

o,p-DDT - ---- = 0

p,p'-DDT - $20 - \frac{50 - 47}{1.44} = 18$

Total quantitation points = 65

Total laboratory score 60 + 65 = 125 (of a possible 200 points)

Tables 2-7 through 2-9 show Relative Performance Rankings for groups of laboratories on check samples of different types. Table 2-7 shows rankings for the laboratories reporting the data in Table 2-2. Laboratories with scores over 190 are considered to have demonstrated generally acceptable performance with some possible minor problems. Scores between 150 and 190 indicate definite problems which should be quickly resolved. Those with scores below 150 should suspend all routine pesticide analytical work pending the resolution of very serious problems in both identification and quantitation, the effects of which place in doubt all routine analytical data output of the laboratories. The laboratories are to initiate corrective action immediately based on the general and individual critiques received and personal consultations with the coordinating laboratory. The remaining portions of the original check sample can be used as a reference standard material to internally evaluate improvement before receipt of a new check sample to again test laboratory performance.

Each set of performance ranking data must be carefully appraised by highly skilled, experienced residue analysts in the coordinating laboratory before deciding upon what, if any, action should be taken based on the results. For example, Table 2-8 shows ranking data for 17 laboratories analyzing a fat sample (results reported in Table 2-5) and Table 2-9 a blood analysis performed by 16 laboratories. Examination of the scores for the fat sample indicates a significant breaking point between laboratories with 185 or more points and those with 168 or lower. Reference to Table 2-5 shows that those below the break point had readily apparent problems and these four laboratories received corrective critiques. To the contrary, all rankings for the blood analysis were 192 or greater, and all laboratories were considered to have turned in acceptable performances, even those with the poorest relative scores.

2I PRIVATE CRITIQUES

As already mentioned, laboratories with significant analytical problems receive added assistance in the form of a private, individual critique of their results reported for a check sample. The content of this critique depends upon the problems that are obvious from a careful analysis of the submitted results and might include comments on incorrect standards, instrumental factors, calculation errors, poor choice of materials or parameters, etc.

2J PROGRESSION OF PERFORMANCE

During the early years of the Interlaboratory Quality Control Program results were expectedly poor. Methodology, equipment, and reagents were a matter of individual laboratory preference, and the first priority was development of uniform methodology and standardization among all laboratories.

TABLE 2-7

RELATIVE PERFORMANCE RANKINGS

CHECK SAMPLE NO. 26, MIXTURE IN SOLVENT

Lab. Code Number	Compounds Missed	False Identifications	No. of Rejects 1/	Total Score 2/
161.	0	0	0	198
137.	0	0	0	198
135.	0	0	0	197
162.	0	0	0	197
87.	0	0	0	197
113A.	0	0	0	197
113.	0	0	0	196
85.	0	0	0	196
48.	0	0	0	195
130.	0	0	0	195
66.	0	0	0	195
73.	0	0	0	194
72.	0	0	0	194
84.	0	0	0	192
89.	0	0	0	192
88.	0	0	1	189
83.	0	0	0	189
96.	0	0	2	187
97.	0	1	0	181
164.	0	1	4	169
68.	1	0	1	168
92.	1	0	0	168
93.	0	1	2	164
90.	1	0	2	159
53.	1	0	1	158
163.	1	1	0	157
95.	2	0	0	146
160	2	1	0	133
45.	0	0	6	128
71.	3	0	3	127
52.	2	1	2	123
47.	3	0	1	115
69.	4	0	2	84
54.	4	4	4	25

1/ Values outside confidence limits

2/ Total possible score 200 points

TABLE 2-8

RELATIVE PERFORMANCE RANKINGS - CHECK SAMPLE NO. 21, FAT

Lab. Code Number	Compounds Missed	False Identifications	No. of Rejects 1/	Total Score 2/
15.	0	0	0	198
16.	0	0	0	198
8.	0	0	0	198
25.	0	0	0	197
7.	0	0	0	195
4.	0	0	0	193
26.	0	0	0	191
33.	0	0	1	191
5.	0	0	0	191
34.	0	0	1	189
11.	0	0	1	188
9.	0	0	1	187
31.	0	0	0	185
6.	1	0	0	168
1.	1	0	0	168
14.	1	0	0	167
24.	0	1	3	165

1/ Values outside confidence limits

2/ Total possible score 200

TABLE 2-9

RELATIVE PERFORMANCE RANKINGS - CHECK SAMPLE NO. 23, SERUM

Lab. Code Number	Compounds Missed	False Identifications	No. of Rejects 1/	Total Score 2/
1.	0	0	0	198
2.	0	0	0	198
3.	0	0	0	197
4.	0	0	0	197
5.	0	0	0	197
6.	0	0	0	197
7.	0	0	0	196
8.	0	0	0	196
9.	0	0	0	196
10.	0	0	0	196
11.	0	0	0	196
12.	0	0	0	195
13.	0	0	0	194
14.	0	0	0	193
15.	0	0	1	192
16.	0	0	1	192

1/ Values outside confidence limits

2/ Total possible score 200

As an example of the improvement attainable from such a program, the recovery and precision results on interlaboratory fat check samples for a group of human monitoring laboratories over a period of years are shown in Table 2-10. The method used in the first year was based on gas chromatography of a concentrated tissue extract without cleanup (1). Although the method was fairly rapid and simple, it was discovered that the GC column and detector became rapidly contaminated by repeated injection of uncleaned samples, and the check sample results proved the method was unsuitable for routine use by a laboratory network. Not only was precision poor as measured by the RSD, but the spread from minimum to maximum recoveries for several compounds was extremely wide, and mean recoveries were generally far from correct.

Conversion was made to a procedure including cleanup of the extract by acetonitrile/petroleum ether partition and Florisil column chromatography (2), resulting in significant improvement in not only precision (Sample 9, Table 2-10) but in accuracy as well. After several months' experience with the method, results on another check sample (Sample 11, Table 2-10) were even better, and with continued participation in the program, the laboratories are making still further progress in their performance as the latest (1974) figures show.

The results in Table 2-11 show progression of laboratory performance on interlaboratory blood check samples between 1968 and 1974. In the beginning the direct hexane extraction method of Dale _et_ _al_. (3) was adopted but was found to yield poor interlaboratory precision. Sample 10 was analyzed by a triple extraction modification of this procedure which also proved inadequate. The later samples were done with the currently recommended Thompson and Walker (4) extraction method, which utilizes a constant speed mixer (Subsection 7A). The results of the blood check samples illustrate again the dual value of the Interlaboratory Control Program in upgrading laboratory performance and in identifying weak analytical methodology.

2K STATISTICAL TERMS AND CALCULATIONS

a. Accuracy and Precision

Precision refers to the agreement or reproducibility of a set of replicate results among themselves without assumption of any prior information as to the true result. Precision is usually expressed in terms of the _deviation_, _variance_, or _range_. _Accuracy_ is the nearness of a result or the mean of a set of results to the true value. Accuracy is usually expressed in terms of _error_, _bias_, or _percentage_ recovery.

Good precision often is an indication of good accuracy, but one can obtain good precision with poor accuracy if a _systematic_ (_determinate_) _error_ is present in the method used. Systematic errors are either

TABLE 2-10

PROGRESSION OF RESULTS
FAT CHECK SAMPLE

Interlaboratory Sample Number	Year	No. of Labs	No. of Compounds	Average Recoveries, %	Average RSD, %
3	1967	15	7	**	50
9	1968	21	7	**	38
11	1969	19	7	108	24
21*	1972	16	7	89	19
24	1973	14	7	95	14
28	1974	10	7	96	12

*Complete data given in Table 2-5

**Unspiked samples for which precise pesticide levels were unknown

TABLE 2-11

PROGRESSION OF RESULTS

BLOOD CHECK SAMPLE

Interlaboratory Sample Number	Year	No. of Labs	No. of Compounds	Average Recoveries, %	Average RSD, %
6	1968	22	6	*	36
10	1969	20	5	*	29
16	1970	22	4	*	21
17	1971	20	4	*	17
22	1972	17	4	96	14
23	1972	17	4	91	12
25	1973	18	4	100	13
27	1974	15	3	*	16

* Unspiked samples for which the actual pesticide levels were not known

positive or negative in sign. The other general classification of errors in analysis is indeterminate (random) errors. These are errors inherent in the analytical methods because of uncertainties in measurements. An example is the measurement of the height of a gas chromatographic peak with a ruler, which even the most careful analyst can measure only to the nearest one mm. Indeterminate errors are random, that is, they are just as likely to be positive as negative. For this reason, the average of several replicate measurements is always more reliable than any of the individual measurements. Although random errors are unavoidable, determinate errors can be corrected once their cause is located.

Standards of accuracy and precision are not the same for a residue analysis as for a macro analytical method such as a titration, for which a precision and accuracy of 1-5 parts per thousand is usually expected of an experienced analyst. The analysis of technical pesticide products is also a macro method for which accuracy and precision are fundamental factors, and the measurement step (usually internal standard GC or LC) must be carried out with this in mind. In contrast with macro methodology, residue analysis involves the assay of nanogram or lower amounts of pesticides, and with the extensive cleanup and great amount of experimental manipulation required, procedures are considered adequately quantitative when values ± 15-20 percent or better are obtained on recovery samples fortified at ppm levels, ± 30 percent at ppb levels. One authority has suggested that a coefficient of variation of less than 40 percent is acceptable for precision between laboratories for a trace analytical method.

Absolute error is the difference between the experimental result and the true value. Relative error is absolute error divided by the true value and multiplied x 100 to yield percent relative error or x 1000 to yield parts per thousand relative error. As an example, an absolute 0.2 µl error in injection of a sample for GC corresponds to $\frac{0.2 \times 100}{1.0} =$ 20% for a 1.0 µl sample but only $\frac{0.2 \times 100}{5.0} =$ 4% for a 5.0 µl sample. It is explained later in Section 3 that low sample injection volumes are to be avoided because of high potential errors. Bias is defined as the mean of the differences (having regard for signs) of the results from the true value.

b. Significant Figures

The uncertainty of a piece of data is assumed to lie in the last digit recorded, and unless qualifying information is given this last digit is assumed uncertain by ±1. If the height of a GC peak is reported as 10.0 cm, the absolute uncertainty is +0.1 cm and the relative uncertainty is $\frac{0.1}{10}$ x 100 = 1%. Likewise, one should always be sure to record all certain figures plus one uncertain figure in a measurement, these figures being designated as significant figures.

Only significant figures should be used in recording and calculating analytical results. If the value 12.3 mg/g is reported for a pesticide analysis, the 12 should be certain while the 3 is more or less uncertain. Good judgment on the part of the analyst is required to decide on the proper number of figures so that significant digits are not lost or non-significant ones retained. All numbers written after the first real number are considered significant. The numbers 1.23, 12.3, and 123 all have three significant figures. Zeroes can cause some problems and should be paid special attention. Zeroes written before the first real number are not significant but merely serve to locate the decimal place. Therefore, the numbers 0.123, 0.0123, and 0.00123 all have three significant figures; the number 0.1012 has four significant figures since the second zero follows the first real number (in this case 1) and is, therefore, significant. All terminal zeroes following a decimal point are significant. For example, 9.800 g indicates a weight of 9.8 grams accurate to the nearest 1 mg, four significant figures. The number 10,100 should indicate five significant figures, but terminal zeroes in a whole number must be considered with suspicion because the proper rule is not carefully followed. If the value 10,100 mm indicates that measurement was made to the nearest 1 mm, the absolute uncertainty is ± 1 mm and the relative uncertainty $\frac{1}{10{,}100} \times 100 = 0.01\%$. If the measurement was actually made only to the nearest 0.1 meter and the final zeroes only indicate the magnitude of the number in mm, the number would better be written in exponential form, 1.01×10^4 mm, to indicate an absolute uncertainty of $\pm 1 \times 10^2$ mm and a relative uncertainty of $\frac{1 \times 10^2}{10{,}100} \times 100 = 1\%$.

Significant figures should be properly retained when performing mathematical operations. Simplified rules which serve in most cases are as follows. In addition or subtraction, the answer has as many decimal places as the number with the fewest decimal places. For example:

$$\begin{array}{rcr} 90.7 & \text{---------------} & 90.7 \\ 8.81 & \text{---------------} & 8.8 \\ +\ 0.551 & \text{---------------} & 0.6 \\ \hline & & 100.1 \end{array}$$

Inspection of the three numbers to be added indicates the answer can have only one decimal place. Each number is initially rounded off to one decimal place and then the sum is taken. Note that the correct answer has four significant figures (even though each number added had only three) but only one decimal place. Rounding off is done by rounding the last retained digit up if the discarded digit is greater than or equal to 5; the last digit is retained unchanged if the discarded digit is less than 5. For multiplication and division, the answer can have no more significant figures than the number with the fewest significant figures. For example, in calculating the ng of pesticide represented by an unknown GC peak by comparison with the area of a standard peak, the formula $ng_u = ng_s \frac{area_u}{area_s}$ is used if response is linear over the range in question. If 1.0 ng standard gives a peak of 9.0 cm height (measured to the nearest 1 mm) and the unknown peak height is 12.0 cm, the ng of unknown is $1.00 \times \frac{12.0}{9.0} = 1.3$ ng with only two significant figures reportable. If an analysis is based on peak areas calculated by the usual formula height x width at one-half height, a width of less than 10 cm measured only to the nearest one mm limits the area and the calculated amount of pesticide to two significant figures.

c. Average

The average or mean ($\overline{X}$) of a set of n values is calculated by summing the individual values and dividing by n:

$$\overline{X} = \frac{\Sigma Xi}{n}$$

d. Range

The difference between the highest and lowest values in a group.

e. Standard Deviation and Variation

Standard deviation (s) of a sample of n results is calculated by use of the equation:

$$s = \left[\frac{\Sigma Xi^2 - \frac{(\Sigma Xi)^2}{n}}{n-1} \right]^{1/2}$$

Variance is equal to s^2. Relative standard deviation (RSD) or coefficient of variation (CV) is the standard deviation divided by the mean and multiplied by 100 (percentage) or 1000 parts per thousand. σ is the standard deviation for a very large set of data, calculated by the above equation with n rather than n-1 in the denominator. Precision is increased (value of s reduced) by increasing the number of replicate analyses, enabling one to determine with greater statistical confidence that the true mean lies within certain limits about the experimental mean or to reduce the interval at a certain confidence level. Confidence limit or interval is defined as:

$$\mu = \overline{X} \pm \frac{ts}{\sqrt{n}}$$

where μ is the true mean, $\overline{X}$ is the experimental mean, and t is a value obtainable in tables for different percentages of confidence and numbers of trials (n). Values of t increase as percentage confidence desired increases and decreases for more replicates.

The EPA Quality Assurance personnel have applied the following test for determining "outlier" values in check sample data, which, if left in, would exert a significant effect on the overall data:

(1) Compute the mean and the standard deviation of the entire data set.

(2) Compute the absolute value of the arithmetic deviation from the mean of all values in the data set.

(3) Establish the correct factor to be used in the mathematical expression in the next instruction from the following table.

Number of data points (n) in the data set	Factor
5	1.65
6	1.73
7	1.81
8	1.86
9	1.91
10	1.96
12	2.04
14	2.10
16	2.15
18	2.20
20	2.24
25	2.33
30	2.39
40	2.49

(4) If the absolute value of the arithmetic deviation from the mean for any number in the data set is greater than the factor times the standard deviation of the entire data set, the number is rejected as lying outside a reasonable data set. The percent confidence interval for the retained values would be given by:

$$\left(1 - \frac{1}{2n}\right)100 = \%$$

This Fitness Test has proven to be practical and reasonable over many years with round robin interlaboratory blind sample exercises wherein proven methodology is used. It is based in part on Chauvenet's criterion as described by Hugh D. Young (5). Individual statisticians disagree on the best test for rejection of questionable results, and no claim is made for the rigorous statistical validity of the method described in this subsection.

g. Total Error

Total error is a method proposed by McFarren *et al*. (6) for combining precision and accuracy in one reporting expression:

$$\text{Total Error} = \frac{\text{Absolute Value of the Mean Error} + 2s}{\text{True Value}} \times 100$$

where s = standard deviation. In general, Total Error values $\leq$ 25 percent are considered excellent, $\leq$ 50 percent acceptable, and >50 percent unacceptable.

Specifically, in the interlaboratory control program, Total Error is calculated from the following equation:

$$\text{Total Error} = \frac{x + 2s}{y} \times 100$$

where x = the arithmetic deviation of the overall mean obtained for a given pesticide from its known formulation value (the absolute value of the mean error), y = the formulation (true) value, and s = the standard deviation. A discussion of this equation has recently been published (7), indicating it may unnecessarily downgrade a considerable portion of results. Alternate equations are recommended which rigorously meet McFarren *et al*.'s 25 or 50 percent criterion with at least 95 percent confidence. These equations are:

$$T = \frac{x + 1.7\,s}{y} \times 100$$

to be used when $x/s > 0.3$ and up to 44 results are available

$$T = \frac{x + 1.8\,s}{y} \times 100$$

when $x/s = 0.3 - 0.15$ and number of results are 45-170

$$T = \frac{2\,s}{y} \times 100$$

when x is not significantly different from zero with 95 percent confidence.

h. Numerical Conversions

1 g = 1000 mg
1 mg = 1000 µg
1 µg = 1000 ng
1 ng = 1000 pg
1 pg = 10^{-12} g
1 ml = 1000 µl
1 µl = 10^{-6} l

2L REFERENCES

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(5) Young, H. D., Statistical Treatment of Experimental Data, Chapter 10, McGraw Hill, 1962.

(6) McFarren, E. F., Lishka, R. J., and Parker, J. H., Anal. Chem., 42, 358 (1970).

(7) Midgley, D., Anal. Chem., 49, 510 (1977).

2M ADDITIONAL SOURCES OF INFORMATION ON PESTICIDE QUALITY ASSURANCE PROGRAMS

(1) Burke, J. A., and Corneliussen, P. E., Quality Assurance in the Food and Drug Administration's Pesticide Residue Analytical Laboratories, Environ. Qual. Saf., Suppl., 3 (pesticides), 25-31 (1975).

(2) Egan, H., Methods of Analysis: An Analysis of Methods, J. Ass. Offic. Anal. Chem., 60, 260-267 (1977).

(3) Eiduson, H. P., Applications of Tolerances, Standards, and Methods in the Enforcement of the Food, Drug, and Cosmetic Act, J. Chem. Inf. Comput. Sci., 17 (2), 102-105 (1977).

(4) Eiduson, H. P., Laboratory Quality Assurance, Bulletin of the Association of Food and Drug Officials, pp. 151-156, 1976.

(5) Youden, W. J., and Steiner, E. H., Statistical Manual of the Association of Official Analytical Chemists - Statistical Techniques for Collaborative Tests, published by the AOAC, PO Box 540, Benjamin Franklin Station, Washington, DC, 1975, 88 pp.

Section 3

INTRALABORATORY QUALITY CONTROL

3A PURPOSE AND OBJECTIVES

The intralaboratory control program is a continuing, systematic, inhouse regimen intended to ensure the production of analytical data of continuing high validity. Several of the program objectives are parallel to those given in Section 2 for the interlaboratory program:

a. To provide a measure of the precision of analytical methods.

b. To maintain a continuing assessment of the accuracy and precision of analysts within the laboratory group.

c. To identify weak methodology and provide a continuing source of research problems aimed at overcoming deficiencies.

d. To detect training needs within the analytical group.

e. To provide a permanent record of instrument performance as a basis for validating data and projecting repair or replacement needs.

f. To upgrade the overall quality of laboratory performance.

The following subsections will treat several integral parts of a high quality intralaboratory quality control program, embracing such areas as the periodic analysis and interpretation of results of spiked reference materials (SPRM's), instrumental maintenance and calibration, and monitoring of the quality of various materials used in the analytical scheme.

3B PURPOSE AND OBJECTIVES OF SPRM'S

In contrast to the interlaboratory check sample program in which one analyst in a laboratory will analyze a sample occasionally sent by the coordinator, the intralaboratory SPRM program provides a continuing measurement of the performance capability of each analyst. Each person can be constantly aware of his strengths and weaknesses, and corrective steps can be undertaken when necessary, before serious problems occur and erroneous data are reported out of the laboratory.

The program involves continual, systematic recovery studies on prepared test samples of each type of substrate routinely analyzed by a laboratory. Each staff chemist conducting routine analyses should participate, and all recovery results are recorded on a table available for examination by the chemist's supervisors.

3C NATURE OF SPRM'S

One possible approach is for a laboratory to prepare its own SPRM's. If the laboratory routinely analyzes animal fat samples, an appropriate check sample may be prepared as follows: Obtain a local bulk sample of 2 lb. or more of fatty tissue, place in a large beaker and warm carefully on a hot water bath to a temperature not above 50°C with intermittent stirring. After a sufficient quantity of liquid fat has been expressed, filter into a second beaker through glass wool (pre-extracted with hexane) held in a glass or porcelain funnel. Heat the filtered fat to ca. 45°C, transfer about one-half to a previously tared flask with standard taper stopper, and reweigh to the nearest 0.1 g. This portion is stored in the stoppered flask in a freezer at -18° to -23°C for later spiking. The remaining half is divided into individual analysis units in small vials or bottles which are also stored in the freezer. The weight of each unit is slightly larger than the intended sample weight. These serve as unspiked SPRM's.

Sufficient analyses are made on the unspiked subsamples so as to be satisfied with the reproducibility of results from the same analyst and among all participating analysts. For verification, the sample may be sent to an outside laboratory with experience in performing the analysis in question. When reproducibility is sufficient to establish a reliable pesticide profile in the unspiked sample, the other half is spiked to produce residue levels approximating or slightly exceeding the levels obtained in routine media. The spiked fat is thoroughly mixed, transferred to small bottles, and stored in a freezer. These spiked samples serve to test the capability of the analyst for recovery of higher pesticide levels.

For both the unspiked and spiked SPRM's, at least a dozen replications of the analysis on the same sample should be conducted by chemists with recognized competence. From this data, the percentage relative standard deviation is calculated and used in construction of control curves as described later in Subsection 3F.

The same basic program outlined for fatty tissue can be followed for other sample materials. If the compound(s) and media are known to be fully stable at room or refrigerator temperature, freezer storage is not required.

The EPA-ETD Interlaboratory Program provides participating laboratories with a sufficient supply of each interlaboratory check sample to serve also as an intralaboratory SPRM for a six month period (Subsection 2D). Laboratories should store the excess material in sample-size portions in a freezer to be withdrawn periodically for analysis along with routine samples. The correct formulation value will be known to the laboratory supervisor when he receives the interlaboratory Summary of Results Table (Subsection 2G) from the coordinator, so that he can compare the results of his personnel with the "correct" values. The advantage of this second approach is that a participating laboratory will have internal SPRM's with reliable results available to them without having to prepare their own samples and establish residue levels and RSD values before they can be routinely used.

Because of their nature, it has not been the practice to treat intralaboratory SPRM's as blinds in the EPA program. A homogeneous, frozen fat check sample in a vial, which is simply dissolved in hexane as the first analytical step, would be difficult to camouflage as a routine fat sample, normally encountered by the chemist as a chunk of adipose tissue requiring initial grinding [EPA Pesticide Analytical Manual, Section 5, A, (1), III, 3]. Likewise, routine blood samples are received as whole blood rather than as the serum form of the check sample. It would be undoubtedly advantageous to devise SPRM's which could be offered to the chemist as a true blind along with his normal sample load, but this has proven a difficult task with fat and blood when it is necessary to prepare a homogeneous sample guaranteed to give a consistent analysis regardless of the portion taken. It might well be feasible for some other sample substrate, such as urine or water.

3D FREQUENCY OF SPRM ANALYSIS

The frequency of SPRM analysis is related to the volume of routine samples run. Laboratories making less than one routine analysis per week of a given substrate should analyze a corresponding SPRM sample with each routine sample, and not less than one SPRM analysis per month even if no routine samples are encountered. Laboratories analyzing one or more samples per week should analyze at least 10 percent as many SPRM samples as routine samples, with a minimum of one per week. For example, if one to fourteen samples are run per week, at least one standard sample should be analyzed each week. If thirty samples are run, one corresponding SPRM sample should be analyzed for each nine samples, or a total of three standard samples. The SPRM is carried through the analysis in parallel with a group of routine samples, giving it no special care or treatment.

In laboratories where more than one chemist performs an entire routine analysis of a given substrate, each individual should run separate SPRM samples. However, if protocol is that routine analyses are handled by a team, e.g., with one chemist preparing extracts and another doing the determination, SPRM samples should be handled in this same normal fashion.

3E RECORD KEEPING

Immediately upon completion of each analysis of an SPRM sample, results are recorded on an Internal Check Sample Form. An example for blood serum is shown as Table 3-1. Data is entered in legible handwriting. Each participating chemist should have access to this record. If significant deviations from the furnished correct (mean) values occur, an investigation is begun at once to determine the reason or reasons.

The chief chemist of each laboratory completes a quarterly report for forwarding to the coordinator and includes in the confidential in-house section (Table 3-2) one copy of each Internal SPRM Report. The coordinator compiles the data from all laboratories and furnishes to each statistical summaries for comparison of results.

TABLE 3-1

RECORD OF ANALYSIS OF STANDARD REFERENCE MATERIAL

Laboratory__________________

____
Media

Analyst or Team__________________

Sample No.	Date	Aldrin	ß-BHC	Hept. Epox.	Diel-drin	o,p'-DDT	p,p'-DDD	p,p'-DDE	p,p'-DDT	

Analyst or Team__________________

Reporting units should be in ppb or ppm. Observe standing instructions for minimum reporting levels.

TABLE 3-2

____
(Date)

SUBJECT: Quarterly Report, Quarter Ending ____________________

TO: Chief, Quality Assurance Section, Analytical Chem. Branch, Health Effects Research Laboratory, (MD-69), Research Triangle Park, NC 27711

FROM: Chief Chemist ____________________ (Laboratory)

During the past quarter we have analyzed the following numbers of routine* samples for pesticide residues:

Blood (multiresidue) ________

Blood (PCP) ________

Blood (Other) ________ (specify)

Adipose Tissues ________

Other Human Tissues ________

Air ________

Soils ________

Stream Sediment ________

Water (multiresidue) ________

Water (Other) ________ (specify)

Urine (alkyl phosphate) ________

Urine (Other) ________ (specify)

Housedust ________

Fish or Shellfish ________

Wildlife ________

Other** ________

Are any spiked SPRM's prepared in-house? Yes No If Yes, list the substrates on the reverse side of this sheet giving the spiking level range of each compound spike.

____
Chief Chemist

*The term "routine" is intended to mean samples of local origin such as donors, autopsies, etc.

**Specify substrates if 10 or more samples were analyzed during quarter.

3F QUALITY CONTROL CHARTS

In addition to recording numerical results of each analysis of an internal SPRM, it may be desirable for each analyst or team to construct a Quality Control Chart. This depends to a great extent on the number of SPRM analyses of a given substrate per week or month. The purpose of this chart is to provide graphic assessment of accuracy and precision for the analysis of each substrate and instant detection of erroneous data. The charts allow quick and easy observation of recovery trends for a particular analysis and have long term value for the self evaluation of analytical output by staff personnel. Another significant value of the charts is that of providing a laboratory administrator with a rapid assessment of the continuing analytical capability of the staff chemists as related to the output of valid analytical data.

The first and very important step in the development of a control chart is the determination of an appropriate value of the relative standard deviation (coefficient of variation) (Subsection 2Ke) for the particular analysis. Stated another way, this is a numerical expression of the potential precision of the method in the hands of competent analysts. Ideally, this value may be obtained by conducting a collaborative study involving at least 8 laboratories. Since the average small laboratory will not be able to carry out this rather involved operation, a satisfactory alternative is for two or more competent chemists of the staff to analyze six replications of the same sample. From these data a reasonable value for percentage RSD may be obtained as shown in 2Ke.

The preparation of the chart is illustrated by the following Figures 3-A and 3-B in which results for a serum intralaboratory SPRM analyzed over a period of three months for p,p'-DDE and p,p'-DDT by chemists in two different laboratories are shown. (Several additional pesticides were also found, but only two are illustrated). Consecutive results are plotted on every second space along the X-axis. The Y-axis contains zero (0), plus (+), and minus (-) lines. The (+) line represents two standard error units (comparable to standard deviations) on the high side from the "correct" answer (the spiking level, or the level found by an experienced analyst in the coordinating laboratory), while the (-) line represents two standard error units (SEU) on the low side. In the case of this sample, it had been previously determined that an appropriate RSD value was 10 percent of the spiking level for each pesticide.

Figure 3-A. Laboratory A control curves for blood SRM, three-month period.

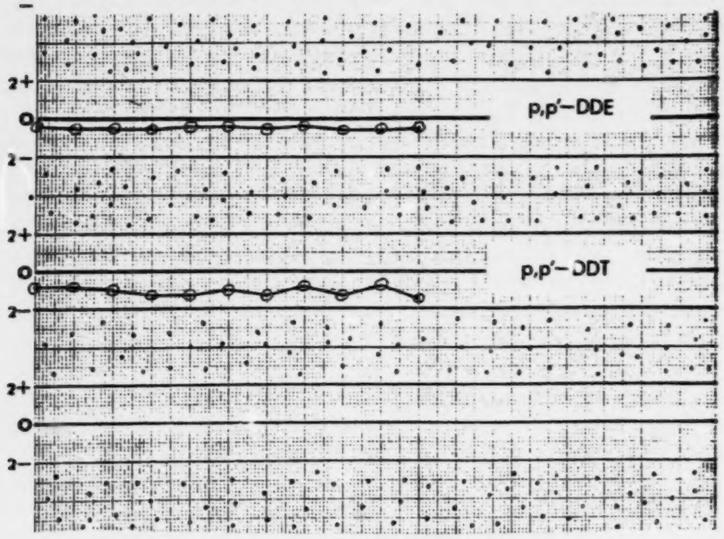
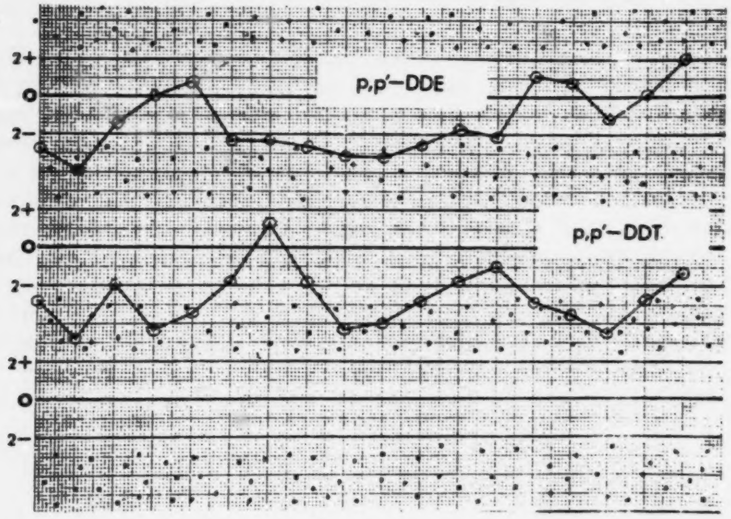


Figure 3-B. Laboratory B control curves for blood SRM, three-month period.



The known formulation or spiking value is subtracted from the experimental value obtained for an analysis of the in-house standard sample to provide a (+) or (-) arithmetic deviation (difference). This difference is then divided by the calculated standard error unit to give the number of standard error units from the correct value. This is the number plotted on the appropriate horizontal line.

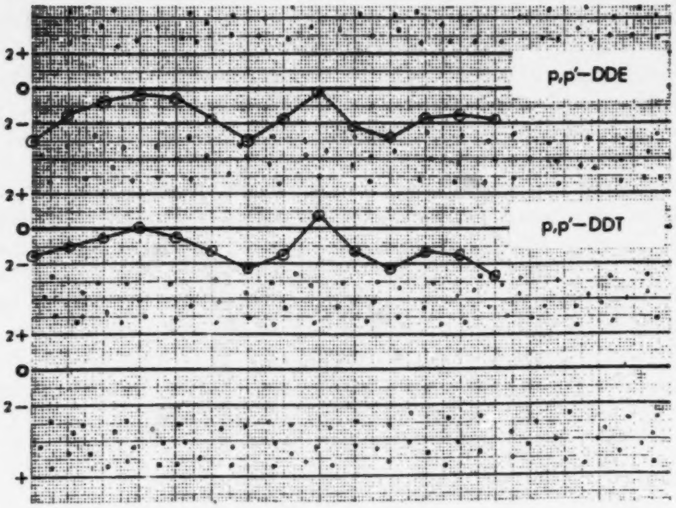
Assume, for example, the first serum SPRM analysis is run during a quarter and a value of 105 ppb is obtained for the content of DDT. The spiking level, however, was 150 ppb. One standard error unit (SEU) is calculated by multiplication of the formulation value by the percent RSD to give a standard error unit that should be valid throughout the life of the specific SPRM: 150 x 0.10 = 15 ppb = one SEU. The difference 105 - 150 = -45 is then divided by 15 to give the number of standard error units to be plotted, in this case -3.0. If the second result is 125 ppb, the second point plotted along the horizontal axis would be calculated as:

$$\frac{125 - 150}{\text{one SEU}} \quad \text{or} \quad \frac{-25}{15} = -1.7 \text{ SEU}$$

When constructed in this way, quality control charts readily show levels of accuracy and precision for repetitive analyses by a given analyst. Figure 3-A demonstrates excellent precision since the results all fall along an essentially horizontal line. Accuracy is good because this line is well within the control area of ±2 SEU, all recovery values being slightly low, probably due to an inherent negative determinative error in the procedure being used. Figure 3-B, on the other hand, demonstrates very poor analytical performance in both accuracy and precision. Nine of the repetitive values for DDE and eleven for DDT are out of the acceptable control range of ±2 SEU.

Control charts also highlight cases where errors are present exerting similar effects on the analyses of several pesticides. The following Figure 3-C, for example, demonstrates rather poor precision and also a distinct correlation in the configuration or shape of the curves for both compounds. This signals some common error proportionately affecting both compounds, most likely the extraction step in this blood analysis.

Figure 3-C. Laboratory C control curves for blood SRM, three-month period.



From time to time, the following question is asked: "What is to prevent an analyst from 'fudging' the control chart points so that his curves will appear significantly better than they should"? This can and has occurred in very rare instances. The alert laboratory administrator, however, should have little difficulty detecting the doctoring of curves. When a chart is submitted that is virtually a straight line such as that for p,p'-DDE in Figure 3-A, his suspicion should be aroused to the extent of personally checking the raw data to either confirm or refute his apprehensions. Furthermore, such an apparently outstanding performance will catch the attention of other analysts in the peer group whose data may look relatively poor by comparison.

In the first sentence of this subsection it was stated that "it may be desirable" to prepare control charts. One main value of the charts is to detect trends. Therefore, if a given SPRM sample is analyzed on an infrequent basis, a chart would serve little purpose as trends would not be evidenced. On the other hand, if a laboratory is monitoring a waterway, for example, for certain pesticides or other organic pollutants, the number of routine samples per month may be 100 samples or more. If the controlling SPRM is analyzed at the recommended minimum rate of one SPRM per 10 samples, this would amount to at least 10 SPRM analyses per month, a number sufficient to justify preparation of the chart.

3G BENEFITS OF THE IN-HOUSE SPRM PROGRAM

Analyzing in-house SPRM's will require a certain amount of man hours during which laboratory personnel cannot accomplish routine, productive analytical work. The time, effort, and expense spent on such a program has proven an invaluable investment, however, in the quality of analytical output in those laboratories involved. For example, chemists from regulatory laboratories are sometimes called upon to testify in a court case based upon their analytical results. If a chemist is armed with high quality analytical assurance data, the validity of his results on the sample(s) in question will be much more difficult to disprove and the case will be that much stronger.

If a laboratory has a correctly functioning intralaboratory control program in effect, the morale of personnel is high, everyone has confidence in the routine data output, and interlaboratory check samples can be taken in stride and handled with little disruption of the normal work schedule. Since a higher volume of uncontrolled analytical data is obviously of much less value than a lower output of reliable results, time and effort must be allowed for each pesticide analytical laboratory that cares about valid results to conduct a proper quality assurance program.

Certain minimum requirements are necessary for the physical plant in which analyses are to be performed. Minimum considerations should include such factors as safety of personnel, reasonable temperature and humidity control, an adequate ventilation system, refrigerated storage areas for samples, facilities for an assembly line layout if large numbers of samples are processed, and an efficient glassware wash area. In addition, all necessary equipment for safety, sample preparation, analysis, and sample and data processing must be available.

The following subsections are intended to highlight a number of in-house factors which can lead to inaccurate analytical data in any laboratory and to present guidelines for avoiding these pitfalls. Further details of many of the areas mentioned will be given in appropriate later sections of this Manual or are covered in the cited sections of the EPA PAM.

3H ANALYTICAL BALANCES

Most laboratories contain balances of two types. Rough triple beam or Dial-O-Gram balances are used for weighing approximate amounts of materials to the nearest 0.01 or 0.001 g. For example, to prepare one liter of a 2 percent solution of NaCl for use in the liquid-liquid partitioning step of the modified Mills, Onley, Gaither Procedure [EPA PAM, Section 5, A, (1)], the required 20 g of salt could be weighed out on one of these rough balances since the concentration of the solution is specified to only one significant figure.

An analytical balance is required, however, for the critical weighing of primary analytical pesticide standards in preparing standard reference solutions. The usual analytical balance has a capacity of 160 g and a capability of weighing to the nearest ±0.0001 g (error and uncertainty), the fourth decimal place being obtained by estimation and therefore the final significant figure recordable (Subsection 2Kb.). This leads to a total accuracy and precision of

$$\frac{0.0002\ g}{0.0200\ g} \times 100 = 1\%$$

in weighing 20.0 mg of pesticide standard by difference (two weighings), as is usually done in preparing primary standard solutions (Subsection 3.M.). This value is quite acceptable considering the other errors inherent in the total analytical scheme.

The accuracy, precision, and sensitivity of the analytical balance should be checked at least once a year by a qualified balance specialist, and the balance should be used properly by all personnel to insure its maintenance in good condition at all times. Since the analytical balance is used to weigh standard pesticides for preparation of solutions upon which all analytical results are based, its importance, and the need for its care and protection, should be obvious. The single pan, direct reading analytical balance which weighs by the principle of substitution is by far the type in widest use today. As compared with the classic, double pan, equal arm balance, the single pan balance is more automatic, convenient, and much faster (although no more accurate or precise), but it is still a very fragile instrument requiring certain precautions in its use. These include the following:

a. The balance should be placed on a heavy, shock proof table or cement block slab built up to convenient height from the floor.

b. The balance is preferably located away from laboratory traffic and protected from drafts and humidity changes.

c. The balance temperature, room temperature, and temperature of the object being weighed should be equilibrated.

d. When not in use the balance beam should be locked, objects removed from inside, and all weights released from the beam.

e. The inside and outside of the balance must be kept scrupulously clean. Never place chemicals directly on the balance pan. Remove spilled chemicals immediately with a brush.

Before using an analytical balance for the first time, the manufacturer's literature should be consulted or instructions obtained from someone experienced in its proper use.

3I PURITY OF REAGENTS

The purity of reagents, solvents, adsorbents, distilled water, etc., is of extreme importance when analyzing samples for residues in the low ppm or ppb range. The electron capture detector senses any electron capturing materials in the injected sample, whether they be pesticides or other impurities. Quite often, extraneous contaminants will give rise to GC peaks that may precisely match the retention characteristics of certain pesticides, even on two or three different stationary phases. A common contaminant of solvents and reagents is di-n-butyl phthalate plasticizer, which can be easily confused with BHC and aldrin in GC with electron capture detection. Construction materials have been suggested as the source of phthalic acid esters, aroclors, and PCBs present in laboratory air and the cause of solvent, reagent, and glassware contamination (1). Sulfur and sulfur-containing compounds can be present in solvents and column materials, as well as in certain substrates (onion, cabbage, turnips), and can give rise to peaks easily confused with pesticides (2).

Commercial solvents designated "pesticide quality" or "distilled in glass" can usually be used without further treatment, but care must be exercised in their storage. For example, it was reported that photochemical reactions can produce compounds from pesticide-grade hexane that are detected by an electron capture detector and interfere with pesticide residue determinations (3). Storage in the dark was recommended to prevent this. Reagent- or technical-grade solvents almost always require distillation by the user in an all-glass still. In any case, each solvent should be checked before use for interference in the analytical procedure by evaporating a portion to provide as great a concentration factor as will ever be employed in any method for which the solvent will be used.

A typical procedure is to concentrate 100 ml of solvent to 1 ml and to inject 5 µl into the gas chromatograph equipped with the detector of choice. Detector response is recorded for at least 20 to 30 minutes. No cloudiness or discoloration should be observed when the volume is reduced, and no GC peaks that would interfere with sample analysis should be produced. Details of the test for electron capture GC are given in Section 3,C of the EPA PAM.

Tests for interfering substances not detected by this procedure but causing pesticide degradation and loss are made by carrying known amounts of standards through the analytical method in the absence of any sample substrate (a complete reagent blank check). Solvents containing oxidants are especially troublesome in causing losses of organophosphorus pesticides, most notably carbophenothion. Acetonitrile and ethyl ether are two common solvents that may require special attention. Impure acetonitrile, the vapors of which will turn moistened red litmus paper blue when held over the mouth of the bottle, should be redistilled. Recoveries of some phosphate pesticides from Florisil columns are low if peroxides are present in ethyl ether eluents. Ethyl ether is tested for the presence of peroxides by adding 1 ml of fresh 10% KI solution to 10 ml of solvent in a clean glass-stoppered flask or cylinder previously rinsed with ether. Shake and let stand for 10 minutes. No yellow color should be observed in either layer. If present, peroxides are removed by extraction with water, after which the 2 percent ethanol normally present in ether and also removed by the partitioning is replaced (EPA PAM, Section 5,A (1)).

Solvent purity for HPLC (4, 5) is at least as critical as for GC, and it is frequently necessary to repurify even the highest quality commercial solvents. Impure solvents can lead to baseline instability, spurious peaks, variable retention volumes, impure recovered fractions, and other problems. Solvent purity is more important in gradient than in isocratic elution. This is especially true of the weaker solvent since more of it passes through the column, and its impurities can be concentrated on the column head. Intentional impurities such as the ethanol stabilizer in chloroform and the antioxidant (UV absorbing) in tetrahydrofuran, as well as HCl or oxidation products in chlorinated hydrocarbons, benzene in hexane, and the afore-mentioned peroxides in ethers, may have to be removed if they interfere. Water content of solvents has an important effect on separations and must be controlled. Tests for solvent purity include recording the UV spectrum in a 5 or 10 cm cell versus air over the dynamic range of the UV detector, spotting residue from evaporation of a large volume for TLC with I_2 vapor visualization, and Karl Fisher titration for water. Antioxidants are easily removed from THF by distillation, but the THF then rapidly oxidizes and must be tested for peroxides with KI as described earlier. HCl is removed from chlorinated solvents and alcohol from chloroform by extraction with water. Water and some other polar impurities are removed from low to moderately polar solvents by column chromatography on activated silica (heated to 175°C), alumina (heated to 300°C), or a molecular sieve. About 2-6 bed volumes should be passed through the adsorbent before replacing it; low cost, larger particle adsorbents which can be dry packed may be used. Water content of solvents is best controlled by preparing dry solvent and blending with water-saturated solvents. Impurities in water are removed by filtration, reverse osmosis, deionization, distillation (neat or from alkaline permanganate), electrolysis, passage through a reverse-phase column (for reverse-phase separations), or combinations of these. Particulates are removed from solvents (especially those cleaned up on an adsorbent column) prior to use in HPLC by passage through a solvent-resistant 0.5 μ membrane filter, and dissolved gases are removed by heating, stirring under a vacuum, or ultrasonic agitation. The composition of solvent mixtures can be altered by prolonged heating or exposure to vacuum. Table 3-3 summarizes some aspects of solvent purity in HPLC as outlined by one instrument manufacturer.

TABLE 3-3

SOLVENT PURITY IN LC [5]

Contaminant	Possible Source	Effect	Removal
Particulate matter (dust, etc.)	During transfer, unclean vessels	May block in-line filters, lodge in pump seals, or accumulate at column head.	Filtration through membrane filter.
Water	Glassware, solvent preparation or manufacture	Variable column activity, k' variation, stability of silicate ester bonded phases.	Drying over molecular sieve or anhydrous sodium sulfate.
Alcohol	Stabilizer in chloroform, impurity in hydrocarbons	Similar to water.	From hydrocarbons, pass through activated silica; from $CHCl_3$ extract with H_2O, dry with Na_2SO_4.
Hydrocarbons (in water)	Organic matter	Baseline instability during gradient elution.	Passage through porous polymer column or C_{18} bonded phase.
Peroxides (in ethers)	Degradation	Oxidation of bonded phase (e.g., $-NH_2$ to $-NO_2$), reaction with sample, column deactivation or degradation (polystyrene-based).	Distillation or passage through activated silica gel or alumina.
HCl, HBr (halogenated solvents)	Degradation	Column degradation esp. bonded phases, UV absorbance (bromide), stainless steel attack.	Passage through activated silica or calcium carbonate chips.
BHT	Antioxidant in THF	UV absorbing.	Distillation.
Dissolved oxygen	Solvent preparation	Degrades polystyrene-based packing, oxidizes β,β'-oxydipropionitrile, may react with sample.	Degas solvent with vacuum or heat.
Unknown UV-absorbing	From manufacture	Baseline instability or drift during gradient elution, high detector background.	Try activated silica or alumina; distillation for organics and recrystallization or passage over ion-exchange column for inorganics.
High boiling compounds	From solvent manufacture	Contaminates collected sample in preparative LC.	Distillation.
Algae in water	Growth during prolonged storage	Can plug in-line filters, column entrance frits.	Distillation from alkaline permanganate or discard.

3J DISTILLATION OF SOLVENTS (6, 7)

Distill reagent grade acetonitrile over reagent grade $AgNO_3$ (3 g/l) with an all-glass fractionating column equipped with a water cooled condenser. Discard about the first 10 percent of the distillate and leave the last 20 percent of the solvent in the flask. Rinse the flask and use fresh $AgNO_3$ and boiling chips for each distillation. Test the distillate for interference. Alternatively, to 4 liters of acetonitrile add 1 ml of 85% H_3PO_4, 30 g P_2O_5, and boiling chips. Allow to stand overnight and then distill from all-glass apparatus at 81-82°C (do not exceed 82°C), discarding the first and last 10 percent of distillate. Distill acetone, hexane, benzene, carbon tetrachloride, chloroform, ethyl ether, isopropanol, methanol, methylene chloride, isooctane, petroleum ether, and ethyl acetate from all-glass apparatus. A technique for recovery of reusable solvent from Kuderna-Danish evaporators has been described (8).

3K MISCELLANEOUS REAGENTS AND MATERIALS

Any other reagents used in the extraction or cleanup steps are also potential sources of contamination. These reagents, such as sodium sulfate (Na_2SO_4), glass beads, sodium chloride, and glass wool, should be pre-extracted with the solvent to be used in the analytical method or another solvent known to remove the potential interferences. For example, Na_2SO_4 is extracted in a reserved Soxhlet apparatus, the thimble of which is pre-extracted before the first use. Methanol followed by hexane or petroleum ether are cycled for several hours each, after which the Na_2SO_4 is dried and stored in a glass container with a glass cap at 130°C in the Florisil oven. Plastic fiber pack liners have been found to contribute PCBs and phthalates to Na_2SO_4 which must be removed by this procedure. Phthalate esters are also removed from sodium sulfate by heating at 600°C for 4 hours in a muffle furnace (FDA PAM, Section 121). Impurities in batches of silicic acid which interfere with separations of pesticides from PCBs were reduced by extraction of the adsorbent with solvent (9).

Filter paper and other reagents and apparatus should be checked by washing through the solvent to be used and injecting a sample, after concentration, into the gas chromatograph. No peaks should appear. Impurities from filter paper were the cause of interfering signals in the GC-alkali flame ionization detector determination of pesticide residues in plants; Soxhlet preextraction of the paper with acetone was recommended (10). Teflon and aluminum foil should be rinsed with an appropriate solvent. Solvents in polyethylene wash bottles can become contaminated with electron capturing and UV absorbing species and should be tested for impurities. Better still, avoid the use of plastic wash bottles and use all-glass ones.

Distilled water can be troublesome, particularly in a procedure where a large volume is used. Such a procedure is the Mills, Onley, Gaither cleanup method for adipose tissue where 700 ml of water is partitioned with acetonitrile, the latter being finally concentrated to 5 ml (a potential contaminant concentration factor of 700/5 = 140). Since the source of contamination in laboratory water is organic in nature, distillation will not be sufficient cleanup if the organic material co-distills with the water. An activated charcoal filtration prior to the distillation procedure has been found to significantly improve water quality. If deionization through a column of ion exchange resin is carried out, an activated charcoal filter should be installed between the column and the distillation equipment to trap any organic impurities eluted from the resin before the water enters the still. For analyses at ppb and ppt levels, distilled and deionized water should be further purified by a double extraction with an appropriate immiscible solvent, e.g., benzene or isooctane, followed by boiling, if necessary, to remove the residual solvent. Aqueous salt solutions such as 2 percent NaCl or saturated NaCl used in some isolation procedures are prepared from properly purified salt and water and then solvent extracted as a further precaution.

Materials in which the initial sample is stored must be given consideration. Polyethylene bags are totally unsuitable for samples to be examined by electron capture GC or TLC because of trace contaminants that may be present. As an example, it has been reported (2) that polyethylene contains a contaminant which reacts with $AgNO_3$ chromogenic reagent, giving a TLC spot close to that of *p,p*'-DDE and having similar GC retention times to *o,p*'-DDE and *p,p*'-DDE. Glass containers with aluminum foil or Teflon-lined caps are generally acceptable as sample containers and for storing purified reagents.

Other examples of problems with reagent contaminants have appeared in the literature. Bevenue *et al*. (11) reported on the contribution of contaminants by organic solvents, glassware, plastic ware, cellulose extraction thimbles, filter paper, and silica gels to water samples causing interference with subsequent GC analysis in the ppb range. Prior to their use, heat treatment of glassware and the silica gels was recommended to eliminate contaminants, while plastic ware and filter paper were excluded from the procedure. Levi and Nowicki (12) found that cloth bags contained residues that were absorbed by cereal grains stored in these bags and gave spurious GC peaks with electron capture detection. The same workers (13) found that Na_2SO_4 , filter papers, solvents from wash bottles, Teflon gaskets, and glass wool produced interfering EC-GC peaks and gave methods for their elimination. Bevenue and Ogata (14) reported on the contribution of extraneous components by high purity, analytical grade basic reagents used for adjustment of pH during isolation steps in the analysis of chlorophenoxy acid esters and ethyl or methyl derivatives of hexachlorophene and PCP in plant and animal tissue and water samples. Baker *et al*. (15) found contamination of acetone with an impurity corresponding to CCl_4 and interfering in the analysis of the latter pesticide (fumigant) by EC-GC. It was shown that this contamination could be caused by CCl_4 in the laboratory atmosphere, possibly arising from the use of aerosol propellent cans for spraying thin layer chromatograms. Trotter and Young (16) found that impurities in $SbCl_5$ reagent caused erratic recoveries of PCBs in perchlorination procedures.

In view of these problems, it is mandatory that reagent blanks be run constantly for each analytical procedure, with final extracts being reduced to the same concentration level normally used for the sample material. A reagent blank involves repetition of the entire procedure without including the sample itself.

3L CLEANING OF GLASSWARE

The residue analytical chemist must be sure his glassware is entirely free from contamination. The cleaning operation generally includes:

a. Soaking and washing in a high temperature (50°C) bath of synthetic detergent (e.g., Alconox) in water.

b. Rinsing with tap water.

c. Rinsing with distilled water.

d. Rinsing with acetone.

Cleaning of glassware used to concentrate samples (e.g., K-D flasks or evaporative concentrator tubes) should include a soak for at least 15 minutes in hot (40-50°C) chromic acid cleaning solution (observe rigid safety precautions) after the tap water rinse to remove all traces of organic material. This soak is followed by *thorough* rinsing with tap and distilled water and then with acetone and hexane. Pipets are washed in the same way, preferably using a commercial automatic or semiautomatic self-contained washing unit.

Large glass items such as beakers and flasks are inverted and suspended to dry in metal racks. Small items such as glass stoppers and bottle caps are wrapped in aluminum foil, dried in an oven, and stored in foil. Pipets are wrapped in bundles in aluminum foil and oven dried.

Clean, dry glassware is stored in a dust-free cabinet. (Stainless steel storage tubes are available for pipets). As an extra precaution, each piece should be rinsed with the solvent to be employed in the analysis immediately before use. As soon as possible after a piece of glassware has come in contact with a sample containing pesticides, it should be rinsed with acetone to remove surface residues. If this is not done, the subsequent soak bath of detergent will pick up the pesticide and may then serve to contaminate all other glassware placed therein.

Details for cleaning glassware are given in the EPA PAM, Section 3, A.

3M ANALYTICAL PESTICIDE REFERENCE STANDARDS

It has often been noted when evaluating chromatograms from interlaboratory check samples that reference standards used in certain laboratories reporting rejected results were undoubtedly inaccurate. (This can be determined by the coordinator by comparing the peak height ratios in the chromatograms from the check sample of precisely known composition against the same ratios from the laboratories' internal standards). The proper preparation and storage of analytical standard solutions is of utmost importance. Since the working, diluted standards may be in use for up to six months, any mistakes in preparation of the concentrated stock solutions or in their dilution would be reflected in the accuracy of analytical results for this entire period. Incorrect standards will result in correspondingly incorrect analytical data even though first class technique is thereafter employed and all laboratory instruments are in perfect operating condition. Even including improperly operated equipment, the greatest single source of quantitative error in GC analysis is undoubtedly inaccurate standard solutions.

Identification and record keeping of reference standard solutions are activities that often receive too little attention in some laboratories. Its importance cannot be overemphasized, particularly in a laboratory concerned with law enforcement. Therefore, the protocol should be formalized and standardized for all staff chemists within the laboratory group. By so doing, it should be possible for any other staff chemist or a supervisor to consult a given chemist's reference standards log book years after an analysis was conducted and readily determine the precise identity and concentration of any standard used in an analysis.

The log book should reflect a complete record of each prepared reference standard solution, starting with the pure primary standard and ending with the final working standard solution. Data that should be documented include weight of primary standard, concentration of all subsequent serial dilutions, and the dates of preparation of all dilutions.

In multiresidue analysis, it is common practice to prepare final working standards as a mixture of pesticides of interest to the laboratory, this subject to be treated in some detail later in this section. Such a mixture should be assigned an identification number and so documented in the log book. The same number should be printed on the bottle label of the mixture and should also be used to identify all reference standard chromatograms during the life of the mixture. A code number such as 26 A_1 might be used, the number 26 indicating the sequential mixture number, the letter A referring to a specified group of component compounds, and the sub 1 to the concentration range.

Sample sheets for maintenance of the reference standards log book are shown in Figs. 3-D, 3-E, and 3-F. These forms are in routine use at the EPA laboratory at Research Triangle, NC.

Details for the preparation, storage, and use of pesticide analytical standards are given in Section 3,B of the EPA PAM. Some important considerations as they pertain to quality control and identification of potential trouble spots are outlined below.

a. Primary Standards

There are no officially recognized pesticide "primary" standards, although in the parlance of the pesticide chemist, analytical grade standards of 99 percent or higher purity are referred to as primary standards. Purities of standards are commonly greater than 99 percent and seldom less than 95 percent but may be lower in some cases. For example, chlordane and toxaphene are available in technical grade with 60-70 percent purity. The percentage of purity must be known in order to apply a correction factor in weighing out the standard for subsequent dilution.

There are several sources of pesticide standards. Most manufacturing companies will supply the analyst with technical grade pesticides and in some cases a small amount of a more highly purified grade. The technical material may be purified by repeated recrystallization and checked for purity by at least two analytical criteria such as elemental composition, IR, NMR, or mass spectrum, melting point, GC trace, or TLC spot pattern. The EPA Quality Assurance Program maintains a pesticide calibration and reference materials repository at its Pesticide Laboratory at Research Triangle Park, NC. This laboratory supplies 100 mg or less of standards of certain pesticides, metabolites, and derivatives, on a discretionary basis as time and resources permit, to nonprofit, government, and university laboratories. EPA publication EPA-600/9-76-012 lists available standards and supplemental data. Purified standards can be purchased from a number of U.S. companies handling chromatographic equipment and supplies and from the National Physical Laboratory, Ministry of Technology, Chemical Standards, Teddington, Middlesex, England.

Concentrated stock standard solutions are conveniently made up at a 200 ng/µl concentration by weighing 20.0 mg of pure standard and diluting to 100 ml. If the primary standard is given as 99.0 percent pure, weigh $\frac{20.0}{0.990}$ or 20.2 mg; if the purity is given as 90.0 percent, the weight will be $\frac{20.0}{0.900} = 22.2$ mg.

Figure 3-D

PREPARATION OF CONCENTRATED STOCK STANDARDS

No._____ Date___/___/___ Chemist____________________

Compound________________ Lot No._______ Purity_________%

Final Gross Wt_________g Dilution Vol.___________ml

*Tare Wt_________g Concentr._____________ng/µl

Net Wt_________g

**Adj. Net Wt_________mg

No._____ Date___/___/___ Chemist____________________

Compound________________ Lot No._______ Purity_________%

Final Gross Wt_________g Dilution Vol.___________ml

*Tare Wt_________g Concentr._____________ng/µl

Net Wt_________g

**Adj. Net Wt_________mg

No._____ Date___/___/___ Chemist____________________

Compound________________ Lot No._______ Purity_________%

Final Gross Wt_________g Dilution Vol.___________ml

*Tare Wt_________g Concentr._____________ng/µl

Net Wt_________g

**Adj. Net Wt_________mg

No._____ Date___/___/___ Chemist____________________

Compound________________ Lot No._______ Purity_________%

Final Gross Wt_________g Dilution Vol___________ml

*Tare Wt_________g Concentr._____________ng/µl

Net Wt_________g

**Adj. Net Wt_________mg

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*If weighing into a beaker, this is the empty beaker weight. If weighing from a dropping bottle, this is the initial weight of bottle and contents.

**Corrected for purity of primary standard.

Figure 3-E

PREPARATION OF STANDARDS OF INTERMEDIARY CONCENTRATION

NO.______ Date___/___/___ Chemist________________

Compound__________________________

Strength of Concentrated Stock__________ng/µl

Aliquot of Concentrated Stock__________ml

Dilution Volume __________ml

Final Concentration __________ng/µl

NO.______ Date___/___/___ Chemist________________

Compound__________________________

Strength of Concentrated Stock__________ng/µl

Aliquot of Concentrated Stock __________ml

Dilution Volume __________ml

Final Concentration __________ng/µl

NO.______ Date___/___/___ Chemist________________

Compound__________________________

Strength of Concentrated Stock__________ng/µl

Aliquot of Concentrated Stock __________ml

Dilution Volume __________ml

Final Concentration __________ng/µl

NO.______ Date___/___/___ Chemist________________

Compound__________________________

Strength of Concentration Stock__________ng/µl

Aliquot of Concentrated Stock __________ml

Dilution Volume __________ml

Final Concentration __________ng/µl

Figure 3-F

PREPARATION OF FINAL WORKING STANDARD SOLUTIONS

No.______ Date___/___/___ Chemist____________________

	Compound	Parent Sol. Number	Conc. of Parent Sol. ng/µl	Aliq. Vol. ml	Dilution Vol. (ml)	Final Conc. pg/µl
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						

No.______ Date___/___/___ Chemist____________________

	Compound	Parent Sol. Number	Conc. of Parent Sol. ng/µl	Aliq. Vol. ml	Dilution Vol. (ml)	Final Conc. pg/µl
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						

No.______ Date___/___/___ Chemist____________________

	Compound	Parent Sol. Number	Conc. of Parent Sol. ng/µl	Aliq. Vol. ml	Dilution Vol. (ml)	Final Conc. pg/µl
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						

Toxicity levels and relative stabilities are important factors which dictate the methods of handling and storing various pesticide standards. Highly toxic pesticides (low LD_{50} values) require special precautions such as wearing disposable rubber or plastic gloves and avoiding inhalation of vapors. The stable organochlorine compounds may be stored at room temperature in tightly sealed containers, while organophosphates, which are subject to a wide variety of oxidations, rearrangements and hydrolytic reactions, should be desiccated in a refrigerator and allowed to come to room temperature in the desiccator before use. If standards are stored in a deep freeze, containers are not opened until warmed to room temperature, or condensed water vapor will be introduced.

b. Concentrated Stock Standards

Secondary standards are liquid solutions of the primary standards. The final concentration of working standard will depend upon its use, e.g., pg range for electron capture GC, ng range for TLC and other GC detectors, and μg range for IR spectroscopy.

For electron capture GC, usually three dilutions of the primary standard are made to arrive at the working standard. An analytical balance capable of weighing to at least 0.0001 g and scrupulously clean glassware are employed. Stable, low toxicity pesticides may be weighed into a small beaker or cupped aluminum foil, transferring solid compounds to the balance with a stainless steel micro spatula and liquids with a pipet or dropper. Crystalline standards weighed on aluminum foil are transferred dry through a small glass funnel into a volumetric flask, the foil and funnel being carefully rinsed with solvent. Standards weighed into beakers are completely dissolved (observe carefully) in a small volume of solvent and transferred quantitatively by rinsing with the rest of the solvent through the funnel into the volumetric flask. Liquid primary standards can alternatively be transferred to a dropping bottle with ground-in stopper; the bottle containing the standard is weighed, an estimated amount of standard transferred directly into a volumetric flask, and the bottle reweighed, the loss in weight representing the net sample weight. This closed dropping bottle technique is mandatory for high toxicity liquid pesticides. Solid primary standards may be weighed (10.0 mg) directly into 50 ml volumetric flasks, which will fit onto the pan of most one pan analytical balances. A procedure for storage and transfer of degradable pesticides under inert atmosphere is given in the FDA Pesticide Analytical Manual, Volume I, Section 132.

It is difficult with any of these techniques to weigh exactly the predetermined amount to obtain all solutions of 200 ng/μl. It is seldom necessary to take the trouble to attempt this, in any case, since the important thing is to obtain a formulation near to that which is desired and to know its exact value. This is calculated by dividing the known weight by the flask capacity. A possible procedure for preparing standards of exactly a certain concentration is to weigh the solid and then add only enough solvent (e.g., the solvent is measured from a pipet, or the solid is weighed into a graduated centrifuge tube and solvent added to the appropriate line) to give the desired concentration.

Benzene will dissolve virtually all pesticide primary standards, gentle heating and stirring on a water bath being occasionally required. Hexane and isooctane are other widely applicable solvents. Dioxane is useful for triazines and methylene chloride for carbamate insecticides.

All concentrations of all standard solutions are preferably stored under refrigeration (-15 to -18°C). Solutions should not be stored in volumetric flasks but transferred to more convenient containers such as inverted ₸ 30 or 60 ml glass stoppered bottles (Corning No. 1560). Organochlorine pesticide concentrated standards are safely kept at least six months. Organophosphate solutions should be remade at least every four months. Pesticide primary standards or solutions should never be stored in the same refrigerator with raw samples awaiting analysis unless extreme care is taken to carefully seal both samples and standards to prevent contamination from spillage or vapors.

c. Intermediate Concentration Standards

It is usually necessary to prepare standards of intermediate concentration by dilution of the concentrated standards and then to prepare working standards by dilution of the intermediate standards. It is impractical and hazardous to prepare the final solution from the concentrated standard in one dilution or to prepare an original secondary standard at a concentration low enough to allow only one subsequent dilution. Some analysts have attempted to make this enormous dilution by aliquoting microliter volumes with a syringe into a volumetric flask. This is extremely poor technique, however, since an error of as little as 0.2 µl in a 5.0 µl transfer will be grossly magnified when a 5 to 10 µl injection of the resulting solution is chromatographed.

The solvents for these standards are usually isooctane, hexane, or methylene chloride, depending upon the solubility of the particular pesticides. The former is preferred, if possible, because of its higher boiling point and therefore smaller chance for solvent evaporation as the solution is used. Changes in volume can occur on opening and closing of the container or even due to volatile solvent leaking through closed containers.

Separate solutions of each compound or a standard mixture can be prepared at this point. The concentration level depends on the response of the detection mode of the analytical procedure in which the standard will be used.

All solutions should be equilibrated to room temperature before any pipeting or diluting is carried out. Volumetric transfer pipets should be used where available, or a Mohr-type measuring pipet in other cases. Be sure to note whether the pipet is calibrated "to deliver" (TD) or "to contain" (TC) and use accordingly. The accuracy of well cared for, properly cleaned commercial Class A pipets and volumetric flasks is such that calibration is not required in order for potential errors from this source to be insignificant.

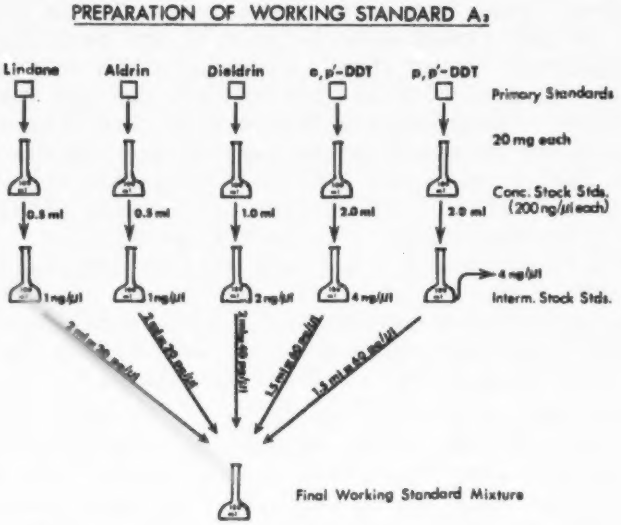
Pipets calibrated to deliver their stated volume should be used if possible. Measuring pipets are calibrated, like a buret, but do not deliver a volume of liquid as accurately or reproducibly as volumetric pipets. The latter are recommended whenever possible for analytical work. Pipets are filled by use of a rubber suction bulb rather than the mouth. After filling and dropping the meniscus to the etched line, no air bubbles should be evident anywhere in the pipet. The outside of the pipet tip is wiped free of liquid and the tip then placed against the inside wall of the vessel to which the solution is to be transferred. The liquid is discharged, keeping the tip against the inside for 20 seconds after the pipet has emptied. The pipet is removed from the side of the container with a rotating motion to completely discharge any drop on the tip. The small quantity of liquid inside the tip is not to be blown out; the pipet has been calibrated to account for this. Only properly cleaned and dried pipets can be inserted into the solution container without fear of contamination or dilution.

Details of storage periods and conditions are given in Section 3, B of EPA PAM. Standard solutions should never be exposed to sunlight or fluorescent lighting for extended periods, either at room or refrigeration temperature.

d. Working Standards

Working standards are generally made up as mixtures, the actual combinations being dependent upon the compounds of interest and the ability of the analytical method to resolve them. Each working standard mixture should be made up at two or even three concentration levels, depending on variations in pesticide concentrations in routine samples. No compound should be present in such concentration that when injected into the gas chromatograph the linear range of the detector will be violated. If p,p'-DDT is present in a standard mixture, neither p,p'-DDD nor p,p'-DDE should be present since these compounds are breakdown products of DDT and their presence or absence is useful for monitoring this degradation. All compounds present in each mixture should be resolved by the working GC columns used in the laboratory. Suggested mixtures and concentration levels of common chlorinated pesticides for laboratories analyzing tissue samples by EC-GC with the recommended columns (Subsection 5L) are given in the EPA PAM, Section 3, B. A typical mixture, diagrammed in Figure 3-G, is prepared as follows: weigh 20.0 mg each of primary standard lindane, aldrin, dieldrin, o,p'-DDT, and p,p'-DDT into separate 100 ml volumetric flasks to prepare concentrated stock solutions of 200 ng/μl each. Transfer, respectively, 0.5, 0.5, 1.0, 2.0 and 2.0 ml of each of these by separate pipets to individual 100 ml volumetric flasks to prepare intermediate stock standards. Transfer 2.0 ml of lindane, aldrin, and dieldrin and 1.5 ml each of o,p'-DDT and p,p'-DDT to the same 100 ml flask to prepare a final working standard mixture containing, respectively, 20, 20, 40, 60, and 60 pg/µl. For other than EC-GC, stock standards of 0.5 mg/ml and working standards from 50-100 to 0.5-1 ng/µl are typical.

Figure 3-G. Serial dilutions of pesticide standard mixture


PREPARATION OF WORKING STANDARD A₂
Lindane
Aldrin
Dieldrin
o,p'-DDT
p,p'-DDT
Primary Standards
20 mg each
Conc. Stock Stds. (200 ng/µl each)
0.5 ml
0.5 ml
1.0 ml
2.0 ml
2.0 ml
1 ng/µl
1 ng/µl
2 ng/µl
4 ng/µl
4 ng/µl
Interm. Stock Stds.
2 ml = 20 pg/µl
2 ml = 20 pg/µl
2 ml = 40 pg/µl
1.5 ml = 60 pg/µl
1.5 ml = 60 pg/µl
Final Working Standard Mixture

e. Storage of Standards

Over the last several years this laboratory has been attempting to evaluate the factors that affect pesticide analytical standard integrity. Briefly the following have been noted:

(1) Solutions of organochlorine and organophosphate standards at working concentration and ambient temperature are stable with respect to compound degradation for up to six months. This tentative conclusion is based on observations to date of 24 organochlorine and 20 organophosphate compounds.

(2) Standard solutions do concentrate by evaporation from glass stoppered volumetric containers at ambient temperature. Hexane evaporates 3.4 times faster than isooctane from closed volumetric containers.

(3) The evaporation rate of isooctane from stoppered volumetrics is independent of the container size. Therefore, the relative loss of solvents is less with the increase in container size. For example, both the 100 and the 10 ml volumetrics in the experiment lost 280 mg of solvent in ten weeks. For the 100 ml container this represented 0.4% of the solvent. But for the 10 ml container this represents 4% of the solvent.

(4) Solvent loss from screw capped prescription bottles or vials at ambient temperature is solvent and seal dependent. Hexane evaporates faster than isooctane. The solvent loss is dependent upon the seal obtained between the cap and the mouth of the bottle. (Some bottles leaked more than others.) If a Teflon cap liner is added to the cap, it must be the correct size. If the Teflon cap liner must be pushed into the cap, it is too large and will crimp. Crimped cap liners will allow solvent evaporation at any temperature, even in the deep freeze. Vial caps with hard Teflon seals allow less solvent evaporation than caps with a soft foam backing behind the Teflon. Solvent loss rate from different screw cap bottles and vials ranged from negligible to significant percentages. This variability was observed within lots of supposedly identical containers.

It has been concluded that analytical reference standards should be kept in relatively large volume isooctane solutions, due both to the slower evaporation rate of the solvent and the slower relative evaporation rate due to the larger volume. When not in use, standard solutions should be refrigerated to reduce solvent loss. The use of prescription bottles is cautiously recommended, provided that the solvent evaporation is monitored. One method is to fill the bottle and mark the meniscus with tape. When the solvent evaporation is obvious, replace the standard with a new one. Organochlorine and organophosphate standard solutions should not be kept longer than six months.

If hexane is used, the recommended replacement time for solutions is 1/3 of the above recommended times.

3N CALIBRATION AND MAINTENANCE OF THE GAS CHROMATOGRAPH AND ACCESSORIES

It is essential that the entire gas chromatograph be maintained in top operating condition if high quality analytical data is to be produced.

In appraising results of interlaboratory check samples, it is clear from data and chromatograms that this is not the case in some laboratories. Section 5 will present details of proper operation of a gas chromatograph. This section will offer guidelines for making routine, periodic checks of equipment to insure continued good operation and minimal down time. Correct procedures for the operations mentioned (e.g., silylation and conditioning of columns, obtaining background profile) will be described in Sections 4, 5, and 9 of this Manual.

Certain checks should be made daily, others on a weekly or monthly basis. Table 3-3 outlines the suggested frequency of such instrumental checks for a chromatograph equipped with an electron capture detector.

It is suggested that a written log be maintained for each instrument, recording the following data:

a. Date of installation and serial number of each detector installed (this will also serve as a record for Atomic Energy Commission inspection).

b. Background current (BGC) profile furnished with the detector under the EPA Interlaboratory Control Program or from the commercial manufacturer.

c. Your own BGC profiles obtained at time of installation of each detector and subsequent profiles (column identity notations should be made).

d. Date of change of pyrometer batteries, if used.

A record should also be kept of each GC column packed and installed in an instrument, logging such information as:

a. Assignment of a column number.

b. Date of packing column.

c. Liquid phase identity and lot number of precoated column packing.

d. Conditioning temperature, flow rate and number of hours,

e. Length and shape of column.

Table 3-4

PERIODIC CHECK LIST FOR GAS CHROMATOGRAPH (EC DETECTOR)

DAILY	WEEKLY	MONTHLY
1. Check response with standard mixture and relate to previous day.	1. Change glass wool plug at column inlet.	1. Clean recorder slide wire with Freon MS-180.
2. Change glass insert sleeve in injection port (end of day).	2. Change septums. 3/	2. Change pyrometer batteries and clean battery contacts with Freon MS-180.
3. Check recorder electrometer zero and noise level at operating attenuation.	3. Run background current profile and polarizing voltage response curve. 4/	3 Check recorder speed at settings in normal use.
4. Check carrier gas flow rate through each column with bubble meter (early morning and afternoon).	4. If endrin is a compound of interest, chromatograph pure standard at a concentration and attenuation that will produce a peak of 50 to 60 percent fsd. 5/	4. Check glass flow system for leaks using "SNOOP". 6/
5. Check temperature of detector, inlet, transfer and column oven. 1/	5. Check for any shifting of column packing resulting in forward movement beyond the bottom of the column exit nut and/or settling in excess of 1/2" from the glass wool inlet plug.	5. Evaluate performance of each column with special standard mixture.
6. Chromatograph standard p,p'-DDT on each working column used at a concentration and attenuation that will produce a peak of 50 to 60 percent full scale deflection (fsd). 2/		6. Check entire instrument for loose connections and frayed wire insulation.
		7. Check all rotameters and flow controllers for proper float action.

1/ Oven temperature should be monitored by an outboard thermometer.

2/ The formation of p,p'-DDD and/or p,p'-DDE indicates on-column breakdown.

3/ This frequency assumes the use of improved silicone rubber. Old type requires more frequent changing.

4/ Daily check may be indicated if large numbers of sample extracts are injected.

5/ The formation of one or two additional peaks indicates on-column breakdown.

6/ Checks should be made whenever a new detector or new tank of gas is installed, or whenever erratic baselines are observed.

f. Background current obtained on newly installed column and subsequent background current profiles during the life of the column.

g. Date of each silylation of column.

h. Compound conversion data, with dates monitored, and percentage of compound breakdown.

i. Monthly, chromatograph the following special column evaluation mixture, recording absolute and relative retention data and efficiency based on the p,p'-DDT peak.

Chlorinated Pesticide Mixture for GC Column Evaluation

Compound	Concentration ng/µl
α-BHC	0.010
β-BHC	.040
Lindane	.010
Heptachlor	.010
Aldrin	.020
Hept. Epoxide	.030
p,p'-DDE	.040
Dieldrin	.050
Endrin	.080
o,p'-DDD	.080
p,p'-DDD	.080
o,p'-DDT	.090
p,p'-DDT	.100

30 ADHERENCE TO OFFICIAL OR STANDARDIZED METHODOLOGY

If reproducible and corresponding data are to be produced on both routine samples and interlaboratory check samples by a group of different laboratories, it is essential that uniform standard analytical methodology be used by all. In the EPA program, this methodology is developed, tested, and collected in the Analytical Manual by the coordinating laboratory of the quality assurance program, in close cooperation with the EPA methods development section. Individual laboratories in the multi-laboratory system are encouraged to suggest improvements in existing procedures, but at no time should any individual summarily introduce method revisions, changes in GC columns, alterations in instrumental parameters, etc., without consultation with the coordinator or authors of methods. Past experience clearly indicates that the vast majority of poor analytical performances on interlaboratory check samples were performed by laboratories deviating in some way from the standard procedures. It is important, therefore, that laboratories adhere to standard analytical methods, but also that they report any problems with them to the coordinator so that these methods can be further researched and improved as experience dictates the necessity. A standard procedure is generally not circulated until such time that reproducibility and precision have been well established. Chemists having troubles with some phase of a standard procedure should search internally for the cause of the difficulty rather than making revisions in the method which cannot be fully studied and statistically evaluated by the individual.

3P IMPLICATIONS OF AN INTRALABORATORY QUALITY CONTROL PROGRAM

An intralaboratory quality control program such as described in the preceding pages requires a good deal of time and effort and does not come cheaply. It is a conservative estimate that around 15 percent of the typical analytical laboratory's resources should ideally be channelled into quality control. The questions often arise, particularly in a smaller laboratory, "Is such a program worth all this effort and expense. What is the return on the investment?"

Each laboratory administration officer must resolve the answers to these questions in light of the impact of his ultimate analytical data. If his laboratory is regulatory in nature, would he feel comfortable going to court to defend the validity of his analytical data? Would his control program hold up under a barrage of cross-examination questions? If the laboratory's work is primarily of a monitoring nature, would he, for example, feel fully confident in advising his superior officials that a given waterway is carrying a pollution load of x micrograms per liter of PCBs?

From observations in the EPA interlaboratory quality control program (Section 2), it can be stated without reservation that laboratories lacking a systematic internal control program more than likely will do very poorly in the analysis of a blind sample. In numerous instances, laboratories joining the program and analyzing a blind for the first time have performed rather badly in contrast to the peer laboratories which have been practicing rigid internal quality control. The practical implication of this, of course, is that analytical data from such loosely controlled laboratories are simply unreliable.

To cite a specific instance, one laboratory joining our program and reporting the results of their first analysis of a spiked water sample reported the presence of *p,p*'-DDE, *p,p*'-DDT, *o,p*'-DDT, heptachlor epoxide, *o,p*'-DDE, and dieldrin. The actual spiking composition was HCB, oxychlordane, *p,p*'-DDE, *p,p*'-DDT, and Aroclor 1254 (PCB). In other words, the analyst found two of the compounds which were actually present, four *which were not present*, and missed three which *were* present.

It takes no great stretch of the imagination to assess the reliability of routine analytical data from this laboratory. Such data would do far more harm than good.

Unfortunately, laboratory administrators are sometimes inclined to regard analytical data as a production commodity, expecting x numbers of analyses to be completed in y length of time with little thought to such ancillary factors as quality control or specific analytical problems related to certain samples. We have no great quarrel with output norms, *provided that* quality control activities are built into the norms. When they are not, analytical data such as those shown above should not be regarded as unusual.

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Section 4

EVALUATION, STANDARDIZATION, AND USE OF MATERIALS FOR PESTICIDE RESIDUE ANALYSIS

4A ADSORBENTS

Cleanup and preliminary fractionation of sample extracts are most often accomplished by chromatographic elution through a column of an active adsorbent. Florisil, a synthetic magnesium silicate, is most widely used for this purpose, but the latest trends indicate that partially deactivated silica gel and alumina, charcoal, and adsorbent mixtures, as well as gel permeation chromatography [1], are becoming increasingly popular.

The activity (adsorptive strength) of adsorbents can be checked by elution of standard dye materials [2], lauric acid, or standard pesticide mixtures through a prepared column. Most materials may be activated by strong heating and some may be activated for a particular purpose by pretreatment with an acid or base (e.g., alumina) or an organic solvent (e.g., charcoal). Deactivation of an adsorbent to a desired level has been achieved by addition of a certain percentage of water.

Florisil has proven to be nonuniform in elution characteristics [3,4] and, therefore, requires careful pretesting of the adsorptive properties of each batch prior to use. One activity of the EPA Health Effects Research Laboratory, Environmental Toxicology Division, Analytical Chemistry Branch Interlaboratory Quality Control Program (Section 2) is furnishing of uniform, standard quality Florisil to other EPA laboratories and to laboratories with direct contracts to conduct environmental monitoring. Procedures specified by the program coordinator and other available standardization methods will be described in the following subsections.

a. EPA Procedures for Handling and Evaluation of Florisil

Details are given in Section 3,D of the EPA Pesticide Analytical Manual. Especially high quality lots of Florisil (calcined at 1250°C) are purchased from the manufacturer in 200-400 lb quantities after favorable evaluation of an advance sample by the coordinator of the interlaboratory program. Upon receipt of the entire lot, another evaluation is made on plugs taken from each polyethylene-lined fiber shipping drum by means of a grain trier. If satisfactory, adsorbent is transferred from the drums to specially cleaned wide mouth glass jars with foil-lined metal screw caps and a capacity for 2 lbs of Florisil.

Evaluation of Florisil for use in a modified Mills, Onley, Gaither procedure is made by heating Florisil in an Erlenmeyer flask overnight or longer at 130°C in an oven which is preferably dedicated to this sole purpose. Heated Florisil is stored at 130°C in the oven with the flask covered by aluminum foil or glass stoppered. Three columns (Kontes 420530, size 241, 25 mm od x 300 mm length) are packed with 4 inch beds of activated adsorbent topped with Na_2SO_4 immediately prior to use, as described on page 6 of the EPA PAM, Section 5, A, (1). Alternatively, the columns may be prepacked, activated, and stored with aluminum foil covers in the oven, and withdrawn a few minutes prior to use.

Two standard mixtures containing a total of 17 chlorinated and phosphate insecticides are prepared at levels of 20-250 pg/μl and 5 ml of each is added to separate columns and 5 ml hexane to the third as a control. Elution is carried out with 200 ml 6 percent diethyl ether in petroleum ether in two 100 ml portions and similarly with 15 percent and finally 50 percent ether-petroleum ether. The six eluates are concentrated and injected for analysis by gas chromatography with an OV-17/QF-1 column capable of resolving the mixtures of pesticides in the fractions.

The percentage recovery for each compound is calculated from the chromatograms of the eluate increments and the original standard mixture. Results are recorded on the standard form shown as Table 4-1. The Florisil is evaluated on the basis of the elution pattern and recovery of the pesticides of interest. All chlorinated insecticides should be recovered in the range 90-105 percent with the possible exception of aldrin, for which recoveries may be low. Some organophosphates, such as carbophenothion, may also yield low recoveries. Ethyl ether should contain 2 percent v/v ethanol as commercially supplied, or if absolute ether is used, exactly 2 percent v/v ethanol should be added to obtain the correct polarity to result in the compound elution pattern shown in Table 4-1. The effects of the ethanol constituent may be observed in the following Figure 4-A wherein three identical mixtures of seven compounds were eluted through three separate but identical Florisil columns. Petroleum ether with no ethanol was used in one column, petroleum ether with the correct 2 percent ethanol in the second column and petroleum ether with 4 percent ethanol in the third column.

A copy of the elution pattern is enclosed with each shipment of Florisil to qualified field laboratories, which should attempt to verify the results. Changes in local conditions, such as packing procedures, temperature, and humidity, can affect the amount of adsorbent or the nature (polarity) of the solvent required for proper elution. Although the specific method outlined evaluates Florisil for use with certain pesticides in a specific procedure, similar methods can be used to pretest different adsorbents for any residue analysis.

TABLE 4-1

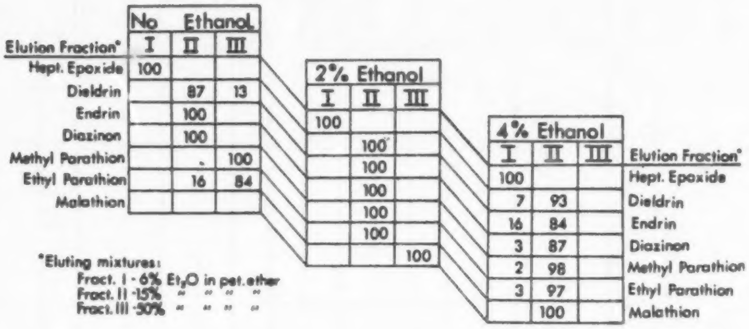
ELUTION PATTERNS AND RECOVERY DATA FOR FLORISIL, LOT # 2961 ,
BY METHOD SECTION 5,A,(1), EPA PESTICIDE ANALYTICAL MANUAL
FLORISIL COLUMN PREPACKED AND HELD IN 130°C OVEN AT LEAST 24 HRS BEFORE USE
RELATIVE HUMIDITY IN LABORATORY 60 %

ELUTION INCREMENTS (ml)

Compound	6% Fraction		15% Fraction		50% Fraction		
	0-100	100-200	200-300	300-400	400-500	500-600	Recovery, %
α-BHC	100						97.3
β-BHC							
Lindane	100						97.3
Heptachlor	100						104
Aldrin	100						97.7
Hept. Epox.	38.9	61.1					108
Dieldrin			22.8	77.2			96.9
Endrin			49.8	50.2			102.3
p,p'-DDE							
p,p'-DDD	100						100
o,p'-DDT	100						105
p,p'-DDT	100						102
Ronnel							
Methyl Parathion			4.1	95.9			86.9
Malathion					94.5	5.5	103
Ethyl Parathion			14.4	85.6			86.6
Diazinon			71.2	28.8			91.7
Carbophenothion	100						46.7

Numerical values represent the percentage of each compound eluting in the given cut.

Figure 4-A. The effects of polarity variation of eluting solvent in Florisil partitioning of 7 pesticides. Absolute ethyl ether mixed with 0, 2, and 4% absolute ethanol.



Elution Fraction*	No Ethanol I	No Ethanol II	No Ethanol III
Hept. Epoxide	100		
Dieldrin		87	13
Endrin		100	
Diazinon		100	
Methyl Parathion		.	100
Ethyl Parathion		16	84
Malathion			

Elution Fraction*	2% Ethanol I	2% Ethanol II	2% Ethanol III
Hept. Epoxide	100		
Dieldrin		100	
Endrin		100	
Diazinon		100	
Methyl Parathion		100	
Ethyl Parathion		100	
Malathion			100

4% Ethanol I	4% Ethanol II	4% Ethanol III	Elution Fraction*
100			Hept. Epoxide
7	93		Dieldrin
16	84		Endrin
3	87		Diazinon
2	98		Methyl Parathion
3	97		Ethyl Parathion
	100		Malathion

*Eluting mixtures:
Fract. I - 6% Et_2O in pet. ether
Fract. II - 15% " " " "
Fract. III - 50% " " " "

b. Florisil Standardization by Lauric Acid Adsorption

For details, see Section 121.3 of the FDA Pesticide Analytical Manual. Florisil is activated and stored as described in Subsection 4Aa. As an alternative, stoppered containers of activated Florisil may be stored in a desiccator at room temperature and the adsorbent reheated at 130°C (unstoppered) after two days.

Standardization by weight adjustment based on adsorption of lauric acid was originally described by Mills [5]. An excess of acid solution (20.0 ml, containing 400 mg) is added to 2.00 g Florisil in a flask, and the amount not adsorbed after shaking for 15 minutes is measured by alkali titration of an aliquot removed from the flask. The weight adsorbed is used to calculate by proportion equivalent quantities of Florisil batches having different adsorptive capacities:

$$\text{Equivalent quantity of Florisil batch required per column} = \frac{110}{\text{Lauric Acid Value of batch}} \times 20\text{ g}$$

$$\text{Lauric Acid Value} = \text{mg lauric acid/g Florisil} = 200 - (\text{ml required for titration} \times \text{mg lauric acid/ml } 0.05N \text{ NaOH})$$

This gross method gives no real indication of the elution pattern to be obtained from a column containing the standardized Florisil.

To verify the value obtained by the lauric acid method and to test for proper elution of organochlorine and phosphate insecticides, 1 ml of a standard mixture containing 1-15 µg of eight compounds is applied to a 22 mm id column containing 4 inches of Florisil (or the weight determined by the lauric acid method) and eluted with 200 ml portions of 6, 15, and 50 percent diethyl ether in petroleum ether. The three fractions are concentrated prior to gas chromatography on an appropriate column. Heptachlor, heptachlor epoxide, ethion, and carbophenothion should elute with good recoveries in the 6 percent fraction; parathion, dieldrin, and endrin in the 15 percent fraction; and malathion in the 50 percent fraction. This mixture is recommended for routine testing since it contains pesticides that give indication of improper elution, poor Florisil, and impure reagents.

c. Deactivated Florisil

Water-deactivated Florisil is required for the Osadchuck et al. multiresidue screening procedure for foods (Subsection 7J). Preparation and standardization is carried out as follows for this method [6]:

(1) Deactivation

Heat 1-2 kg of Florisil in a one gallon jar at 300°C for 8 hours and cool overnight. Add 2 percent (w/w) distilled water and place a screw cap lined with aluminum foil on the bottle. Place the jar in a rotary mixer, tumble for 1 hour, and allow Florisil to stand for 24 hours after mixing.

(2) Standardization

A mixture of dieldrin, malathion, and azinphosmethyl is added to a 2.5 cm id tube filled with 15 cm of deactivated adsorbent. The column is eluted successively with 300 ml portions of 30 percent methylene chloride in hexane, 10 percent ethyl acetate in hexane, and 30 percent ethyl acetate in hexane. Dieldrin should elute in the first fraction, malathion in the second, and azinphosmethyl in the third, with all recoveries greater than 90 percent. Late elution, especially of malathion, which just barely elutes with 10 percent ethyl acetate, indicates insufficient deactivation and the need for more polar solvents. Early elution indicates over-deactivation, requiring less polar solvents for chromatography (i.e., lower percentage of methylene chloride or ethyl acetate).

Comparable standardization is carried out for other methods employing deactivated Florisil.

d. Deactivated Silica Gel and Alumina

Silica gel deactivated with various percentages of water has been successfully used for cleanup and fractionation in many residue determinations. Preparation of 20 percent deactivated adsorbent on a small scale has been conveniently and successfully carried out as follows [7]:

(1) Woelm silica gel is activated for 48 hours at 170-175°C.

(2) Add two ml water to 10 g of adsorbent in a tightly capped Teflon-lined screw top vial.

(3) Mix on a rotary mixer (Roto-Rack™) for 2 hours at setting 8.

Silica gel prepared in this manner can be stored in the capped vial for at least one week with no change in adsorptivity. Standardization is carried out, as above, by packing the required column, adding an aliquot of standardization solution containing the pesticides of interest at a level providing adequate detector response, eluting with appropriate solvents, and examining fractions of eluate by gas chromatography.

Alumina deactivated with water is used in conjunction with silica gel in the Holden and Marsden cleanup procedure (Subsection 7A) and its various modifications (8). This may be prepared in a similar manner by addition of the required percentage of water to alumina previously activated at 800°C for 4 hours.

e. Celite 545

Electron capturing impurities are removed from Celite 545 as follows: slurry with 6 M HCl while heating on a steam bath, wash with water until neutral, wash with several solvents ranging from high to low polarity, and dry. Impurities interfering with phosphorus-selective detectors are removed by heating Celite at 600°C in a muffle furnace for a minimum of 4 hours (FDA PAM, Section 121).

f. Charcoal

Charcoal adsorbent is purified as follows: slurry 200 g with 500 ml concentrated hydrochloric acid, and stir magnetically while boiling for one hour. Add 500 ml of water, stir, and boil another 30 minutes. Recover the charcoal by filtering through a Buchner funnel, wash with water until washings are neutral, and dry at 130°C. (FDA PAM, Section 121). As an alternative procedure (9), add 225 g charcoal to 1.2 liters of ethanol-conc. HCl-water (50:10:40) and reflux for one hour. Collect the charcoal on a Buchner funnel and wash with distilled water until pH test paper shows only a trace of acid to be present. Further wash the charcoal with acetone and aspirate until nearly dry. Air dry until odorless (2-3 days) and finally dry in a porcelain dish at 130°C for 48 hours. Store in a tightly stoppered bottle.

g. Magnesium Oxide (Sea Sorb 43)

Slurry 500 g with enough distilled water to cover it in a one liter Erlenmeyer flask, heat with occasional shaking for 30 minutes on a steam bath, and filter with suction. Dry for 12-24 hours at 105-130°C and pulverize to pass a No. 60 sieve. About 10 percent water is absorbed in this procedure. Store in a closed jar (FDA PAM, Section 121; 9).

h. Packing and Elution of Adsorbent Columns

Pack the adsorbent in glass chromatographic columns containing a loose plug of glass wool (coarse porosity fritted glass discs as support are not recommended because of the difficulty of keeping them clean). Columns 300 mm x 22 mm id with or without a Teflon stopcock (e.g., Kontes 420530, size 241, or equivalent) have been widely used for larger scale cleanup, and 7 mm id columns (e.g., Kontes size 22 Chromaflex columns, or equivalent) for small scale chromatography. Add the required amount of dry column packing in increments and gently tap to settle after each addition; then add a layer of granular sodium sulfate (ca. 0.5 inches) on top of the adsorbent. Prewash the column with hexane or petroleum ether, bring the level of liquid to the top of the bed, add the sample and wash it into the bed with several small portions of the first eluent. Collect the various fractions in separate containers.

Carry out the elution with a series of solvents and solvent mixtures of increasing polarity. Select the polarity of the solvent series consistent with the activity (polarity) of the adsorbent and the polarity of the sample. Use the least polar solvent that will elute the pesticides from the adsorbent to minimize co-elution of polar impurities.

The order of polarity for several common solvents is as follows:

Hexane (petroleum ether) - least polar
benzene
ethyl ether
methylene chloride
ethyl acetate
acetonitrile
methanol - most polar

Section 4B

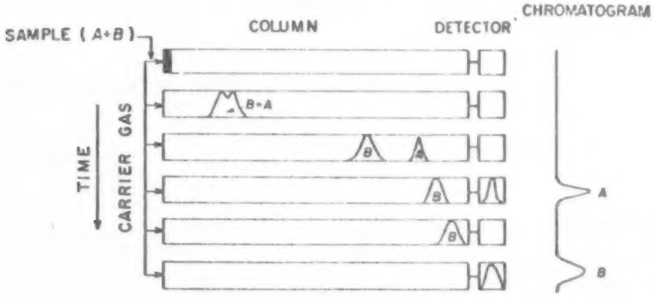
GAS CHROMATOGRAPHY PACKINGS

4B INTRODUCTION AND COLUMN TECHNOLOGY

It is appropriate to reiterate that the column is the "heart of the gas chromatograph". Even though all other modular components of the instrument may be functioning perfectly, a bad column will cause the entire gas chromatographic output to be correspondingly bad. In this subsection, a number of practical operational problems will be discussed; many of these problems have come to light in the interlaboratory quality control program described earlier in Section 2. Some of the operational instructions, fully covered in Sections 4,A and 4,B of the EPA PAM, will be briefly reviewed in this subsection, but only as they relate strongly to the success or failure of the gas chromatographic performance.

A column for gas liquid chromatography consists of a tube filled with a powdered support on which is uniformly coated a liquid, stationary phase. When a mixture of compounds is injected into the gas chromatograph, each compound is swept through the column at a rate that is determined by the interaction of the compound with the stationary phase under the given operating parameters such as temperature and flow rate of carrier gas. If the phase and the parameters are properly chosen, the different compounds will migrate through the column at different rates, and separation will be achieved as diagrammed in Figure 4-B (10).

Figure 4-B. Schematic diagram for elution analysis.



Most applications of residue analysis are carried out with packed columns of this type rather than wall-coated or support-coated capillary (open tubular) columns, although the latter have been used when mass spectroscopy and gas chromatography are used in combination for identification or assay (Subsection 8H).

GC tubes are usually made of borosilicate glass. Copper and stainless steel are best avoided because both can cause decomposition of compounds unless special precautions are taken.

Commercial solid support materials are usually composed of flux-calcined diatomaceous earth which may be treated by acid- or base-washing or silanization. Firebrick, glass beads, and Teflon are other support possibilities. A good support material should be available in narrow and uniform ranges of particle (mesh) size and have a minimum of active adsorption sites for interaction with injected compounds passing through the column, high surface area per unit volume, good thermal stability and mechanical strength. Although greatly improved in recent years, various supports and different lots of the same support are not necessarily equal in surface area or inertness. Adsorption or degradation of a pesticide on the support can affect the relative retention time and response of the compound. It is important to select the most inert solid support possible for pesticide analysis, with additional special treatment being desirable for columns used to determine certain sensitive compounds (Section 4F and 4I). As a general rule, column efficiency increases as the particle size of the support decreases, but a greater carrier gas pressure is required to maintain a given flow through columns with smaller mesh sizes. For most pesticide work, supports with mesh sizes of 80/100 or 100/120 will be satisfactory. The presence of very fine particles, those above the upper limit of each individual mesh range, may cause column inefficiency. If it is likely that particles have been broken during shipment or use, thus increasing active sites and exposing untreated surfaces, check to determine whether the mesh size of the solid support is completely within the expected range.

There are a great number and types of liquid phases commercially available. The choice of liquid phase is usually made on the basis of the polarity of the compounds to be separated. Phases recommended for general use in pesticide analysis are described in Section 5L. Recently, liquid phases have been marketed that are purportedly "equivalent" to previously available phases but with greater thermal stability. It is important to determine whether they provide the same relative retention times.

Important column considerations include efficiency and resolution capability, sensitivity (in relation to the detector), retention, compound elution pattern, stability to heat and injection loading, and freedom from on-column compound decomposition. These will be discussed in light of their effect on day-to-day operation of the column.

4C EFFICIENCY AND PEAK RESOLUTION

Figure 4-C shows the equations used for calculating column efficiency (in theoretical plates) and the resolution (R), or degree of separation between peaks, from a chromatogram. A numerical value for efficiency in itself, is of little practical import. However, efficiency is generally synonymous with peak resolution, and this is of considerable importance to the chromatographer. Figure 4-D, for example, shows superimposed chromatograms of standard chlorinated pesticide mixtures on two separate 6-foot columns of 2% OV-1/3% QF-1, one (A) with very poor efficiency (740 total plates) and the other (B) with very high efficiency (4,530 plates). It will be observed that on column B, all seven peaks give base-line separation, whereas on the low efficiency column A, poor separation is evident for four of the peaks.

A column efficiency value of 500 theoretical plates per foot for *p,p*'-DDT is considered to be of minimal acceptability in terms of the generally expected peak resolution. A 6-foot column of 3,000 plates will usually provide acceptable resolution of mixtures encountered in residue analyses. Since the absolute retention time of the peak used for measurement has an effect on the calculated N, it is necessary to choose a standard peak such as *p,p*'-DDT for comparison of column efficiency. Column efficiency as measured by this equation is affected by noncolumn factors such as dead-volume in the instrument construction or by any gas leaks.

Figure 4-C. Calculation of column efficiency and resolution.

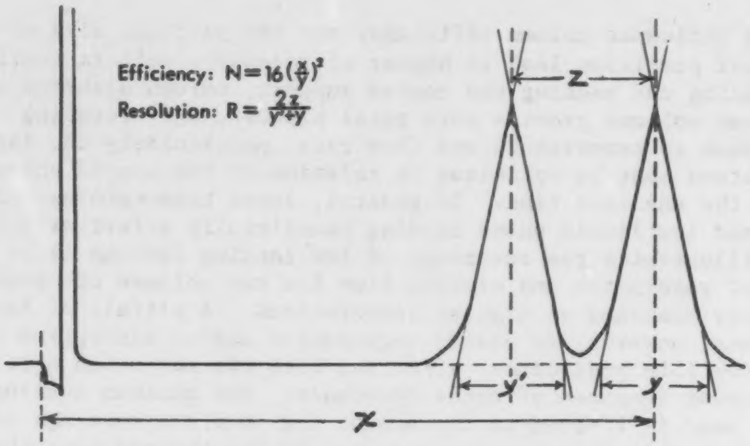
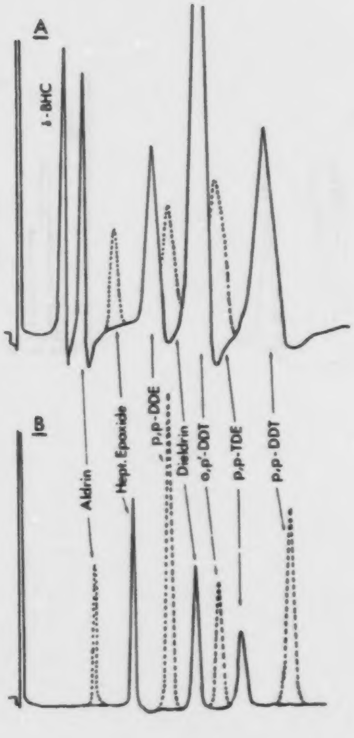
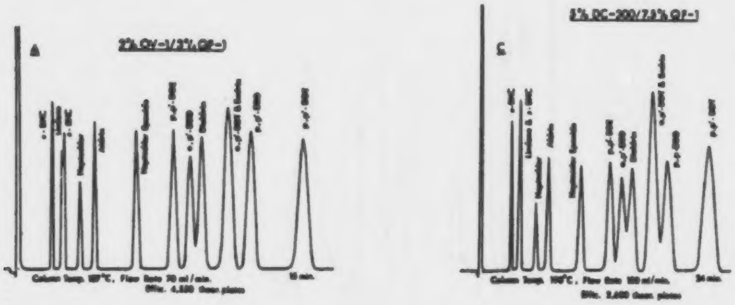

Efficiency: N=16(x/y)²
Resolution: R = 2z/(y'+y)
z
y'
y
x

Figure 4-D. Effect of column efficiency on pesticide resolution.



Factors that influence column efficiency are the particle size of the support (small particles lead to higher efficiency), uniform coating, care in handling and packing the coated support, column diameter and length (longer columns provide more total plates), and operating parameters such as temperature and flow rate, particularly the latter. These parameters must be optimized in relation to the liquid phase loading and the analysis time. In general, lower temperatures and flow rates and low liquid phase loading beneficially affect efficiency. Figure 4-E illustrates the advantage of low loading (column A) by comparison of resolution and elution time for two columns of nearly equal polarity operated at similar temperatures. A pitfall of low-loaded columns, however, is easier degradation and/or adsorption of certain susceptible pesticides, affecting both the retention time and the apparent response of these compounds. The minimum coating that can be used is limited to the amount for complete coverage of the support, usually 1-3 percent, and also by the reduced capacity for sample components.

Figure 4-E. Effect of stationary phase loading on column efficiency.



4D SENSITIVITY AND RETENTION

The same principal factors influence the sensitivity and retention of the column: type and loading of the liquid phase, carrier gas flow rate, column temperature, column length, and particle size of the support. These column parameters influence the sensitivity in that any change increasing the peak height for injection of a given amount of pesticide will thereby increase detector response. The columns recommended in this Manual (Subsection 5L) are designed for adequate resolution consistent with practical elution times, and an absolute retention of 16-20 minutes for p,p'-DDT has been found to approximate these characteristics for a column. This retention range can be obtained by operation of lower load columns (3-6 percent) under such conditions that will produce maximum efficiency. Higher load columns must be operated at elevated temperature and flow rate, and therefore decreased efficiency, to obtain this elution time. Relative retention times are affected only by the nature of the liquid phase and the column temperature. That is, at a constant temperature, the percentage loading of a particular liquid phase can be varied without changing the relative retention of two or more pesticides.

The following bar graph, Figure 4-F, provides comparative sensitivity data on eight GC columns using the 3% OV-1 column as unity for reference purposes. Each column included in the study was operated at its optimum parameters in terms of the achievement of maximum response, efficiency, and a practical retention time.

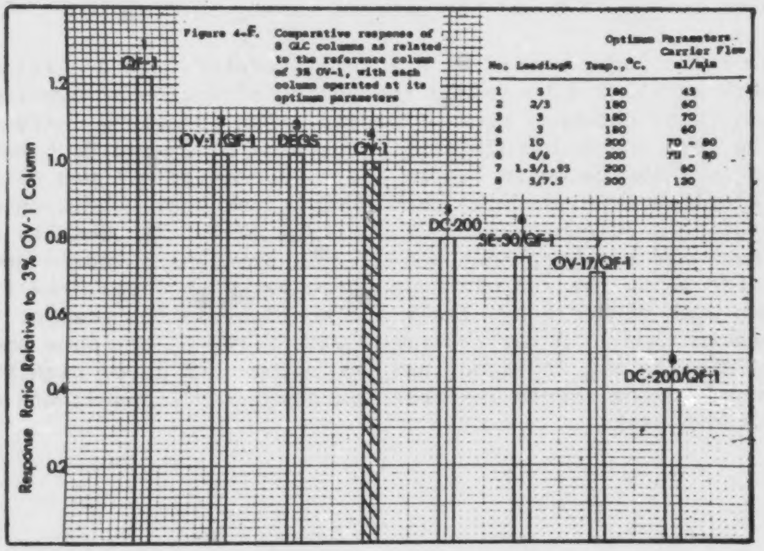


Figure 4-F. Comparative response of 8 GLC columns as related to the reference column of 3% OV-1, with each column operated at its optimum parameters

		Optimum Parameters	
No.	Loading%	Temp., °C.	Carrier Flow ml/min
1	5	180	45
2	2/3	180	60
3	3	180	70
4	3	180	60
5	10	200	70 - 80
6	4/6	200	75 - 80
7	1.5/1.95	200	60
8	5/7.5	200	120

4E COLUMN STABILITY

It is desirable to use columns that are heat-stable or "bleed" resistant and that continue to function properly under injection loading with dirty extract. Liquid phase bleed is evident from a persistently drifting baseline and the inability to obtain a normal level of standing current (Subsection 5C) from an electron capture detector.

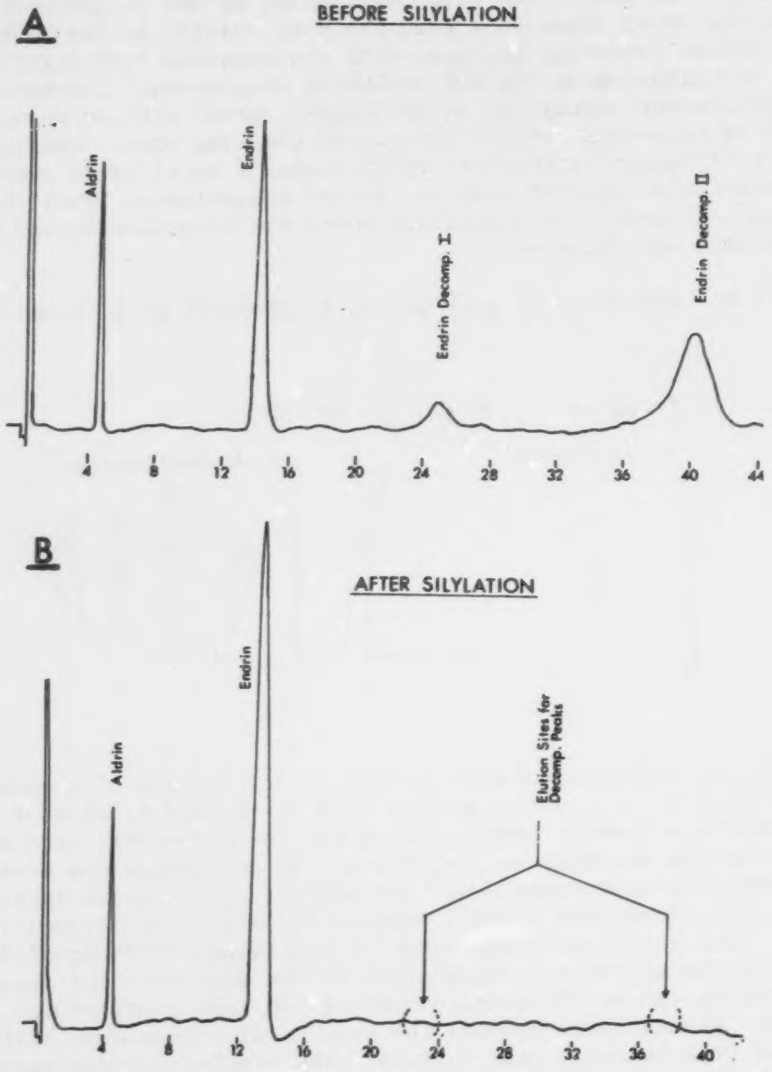
When a succession of "dirty" extracts are passed through the system, the column performance is usually affected. The most prevalent symptoms of injection overloading are depressed peak height response, lowered efficiency and resolution, on-column conversion of pesticides, erratic recoveries, and unsymmetrical peaks (see Figure 4-I).

4F RESISTANCE TO ON-COLUMN COMPOUND DECOMPOSITION

Unless a column is properly prepared, conditioned, and maintained, it can cause such compounds as endrin and/or p,p'-DDT to undergo some degree of decomposition. The main symptom of endrin decomposition is a greatly reduced endrin peak with the formation of one or two additional peaks arising from decomposition products. p,p'-DDT decomposes to p,p'-DDD and, in extreme cases, to p,p'-DDE.

Newly packed columns should be specially treated with a silylating agent such as Silyl 8 to reduce the number of active adsorption sites which can cause decomposition of endrin. The beneficial effect in improving response and minimizing conversion of endrin to breakdown products is illustrated in Figure 4-G. Chromatogram A was obtained for an aldrin-endrin mixture immediately after heat conditioning and equilibrating a column of 1.5% OV-17/1.95% QF-1. It exhibits a small endrin peak and two breakdown peaks. (In principle, endrin could be quantitated using the sum of these three peaks; however, the final breakdown peak elutes very slowly and would cause the analyst to waste considerable time.) After treatment with Silyl 8, the same amount of the same mixture was injected, and Chromatogram B shows significant improvement in the endrin response and complete disappearance of the two breakdown peaks.

Figure 4-G. Reduction in breakdown of endrin resulting from column silylation



Silylation does not always provide such dramatic results. Cases have been noted when no endrin response whatever, either in the form of a main peak or breakdown peaks, was obtained, and silylation did not improve the situation. On the average, however, silylation clearly improves the gas chromatographic behavior of endrin.

DDT breakdown is manifested by the appearance of p,p'-DDD and/or p,p'-DDE on the chromatogram resulting from the injection of pure analytical grade p,p'-DDT which is known to be free of these metabolites as impurities. This problem is associated with overloading of the column packing adjacent to the front glass wool plug,the plug itself, or the glass insert if off-column injection is used, with contaminants from dirty extracts. Figure 4-H illustrates the DDT breakdown phenomenon. Chromatogram A is an aldrin-DDT mixture on an SE-30/QF-1 column with no decomposition, while B shows another column containing the same phase (operated with somewhat different parameters) which caused a total of 25 percent decomposition of the DDT peak to its two metabolites. This chromatogram was obtained in a laboratory where the injection insert had not been changed for three weeks.

Figure 4-H. Breakdown of p,p'-DDT on 4% SE-30/6% QF-1 column.

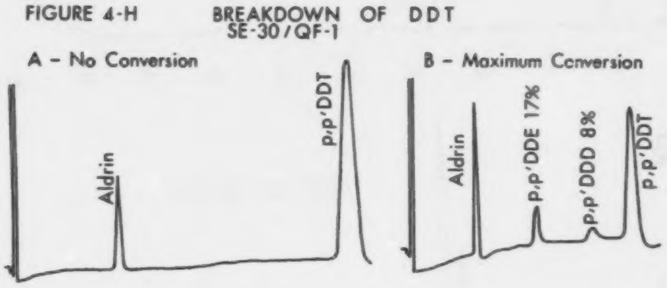
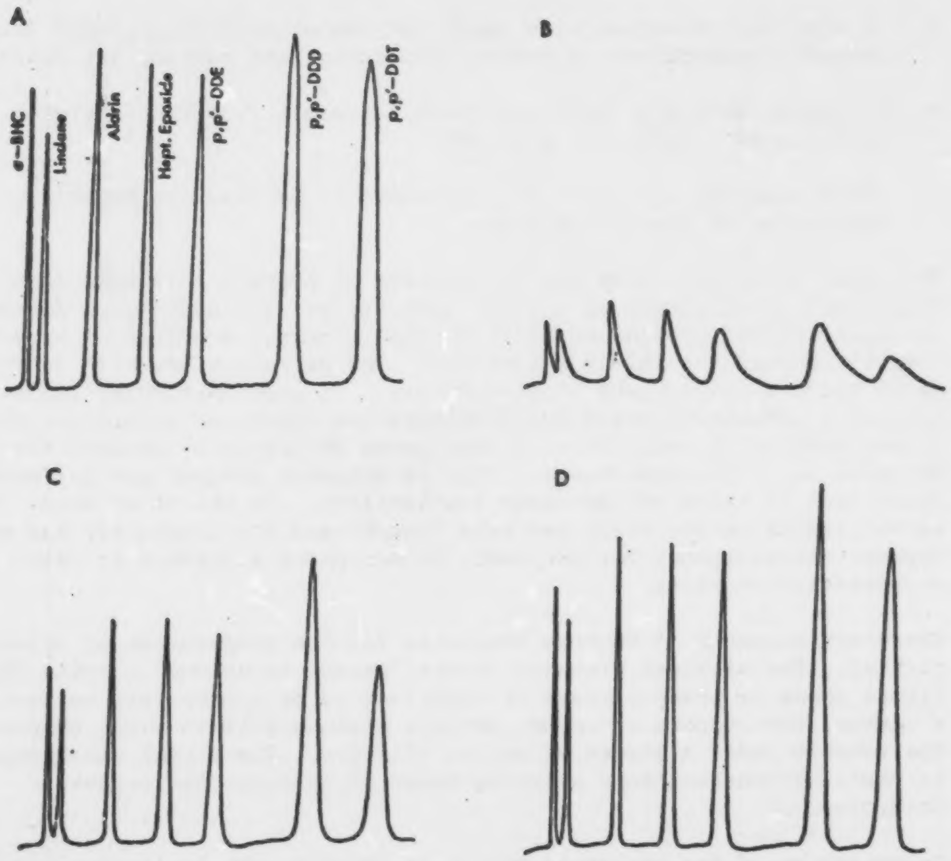


Figure 4-I illustrates an extreme case of overloading of a column of 2% OV-1/3% QF-1. Chromatogram A is from a standard mixture of seven pesticides on a freshly prepared column. The column was then disconnected from the detector so the exit end vented inside the oven. Eighteen consecutive injections were then made of fatty tissue extract after elution with 15 percent diethyl ether-petroleum ether through a Florisil column, each injection containing the equivalent of 25 mg of fat. After 30 minutes the column was reconnected to the detector, the system equilibrated, and an identical volume of the same standard mixture was injected. Chromatogram B shows the results of column overloading: depressed peak heights, peak tailing, peak broadening, and conversion of p,p'-DDT to p,p'-DDD (in actuality, the ratio of these changed from 8:10 to 4:10). A clean Vycor glass insert was then installed in the injection port, the system re-equilibrated for 30 minutes, and another equal volume of standard mixture injected. Chromatogram C shows the dramatic recovery of the system after this single step. Finally, Chromatogram D indicates a complete rejuvenation of the system when the same mixture was injected after overnight purging at normal operating temperature and carrier flow parameters.

This series of chromatograms is striking evidence that damaged columns can often be salvaged by changing the injection insert, forward glass wool plug, and perhaps the first one-half or one inch of column packing. More importantly, properly maintained and monitored columns should provide top performance without problems for many thousands of injections.

Figure 4-I. Chromatograms illustrating column overloading and subsequent rejuvenation.



4G HOMEMADE VS. PRECOATED PACKINGS

The decision whether to make column packings or to buy them precoated confronts every laboratory conducting GC analyses. Prior to 1969 the answer to this question was easy. The precoated supports available from commercial suppliers were so poor in quality that it was necessary to hand-coat packings to obtain satisfactory materials. Since that time, however, several commercial firms have developed the capability to produce high quality packings. Notwithstanding, anyone purchasing this material should do so on specification. As broad guidelines, the following quality criteria are presented:

a. Must meet a column efficiency of a minimum of 3,000 theoretical plates for a column of 183 cm (6 ft) x 4 mm (5/32 in), computation being made on the basis of a peak for p,p'-DDT.

b. A specific pattern of compound elution and peak separation.

c. An absolute retention time range for the elution of p,p'-DDT using specified parameters of column temperature and carrier gas velocity.

d. No appreciable decomposition peaks to result from the injection of pure standard endrin or p,p'-DDT.

e. Final acceptance of each lot purchased to be based on buyer's evaluation at time of delivery.

The final decision of whether to purchase or prepare column packing may depend on the situation in a given laboratory. The successful formulation of column packing in small batches requires a degree of expertise somewhat beyond the purely scientific. The procedure has been described as 50 percent science and 50 percent art. If some particular individual on a laboratory staff has developed the expertise to produce good column packing in small lots, it may prove advisable to prepare the material on an in-house basis. This is somewhat cheaper and far more convenient in terms of immediate availability. On the other hand, if no individual on the staff has this "knack" and the laboratory has no appropriate equipment for the task, it may prove advisable to rely on a commercial supplier.

There are a number of methods available for the preparation of column packing. The simplest probably is the "beaker technique" wherein the liquid phase or phase mixture is dissolved in an appropriate solvent in a beaker, the support is added, and the mixture stirred while evaporating the solvent under a stream of air or nitrogen. The strong disadvantage is that the constant hand stirring tends to fracture the support particles.

An extension of the beaker technique is known as the "filtration technique". The slurry in the beaker comprised of liquid phase, support, and solvent is removed by drawing air through the layer of packing on the filter paper by means of a side arm flask connected to a vacuum source.

The "fluidization technique" is a more sophisticated extension of the beaker technique. The slurry in the beaker is transferred to a fluidizer cylinder (Applied Science Laboratories, Catalog Number 13994) so constructed that a high volume of nitrogen can be blown up through the packing from the bottom of the cylinder, while heat is applied by an element at the base of the cylinder.

In the "rotary vacuum technique" the liquid phase or mixture is dissolved in an appropriate solvent in a small beaker and transferred to a Morton flask (Kontes No. K-295900) with indented sides. The support is added and the flask is placed in a variable heat water bath and connected to a rotary evaporator (Rinco). Mixing and solvent evaporation are carried out by rotating the flask under vacuum with applied heat.

As no preparation technique is presented in the EPA PAM, one method is offered below for the benefit of laboratories which may like to prepare their own packing. While other methods may be equally satisfactory, the rotary vacuum method as detailed here has proved very satisfactory for the production of small batches of GC column packing. The batch size described will provide enough packing to fill three 183 cm x 4 mm columns.

a. Based on a 21 gram total batch size, compute the amount of liquid phase(s) to weigh in 30 ml beaker(s) on an analytical balance.

b. Weigh out liquid phase to two-place accuracy. If making mixed-phase packing, weigh each liquid phase in a separate beaker.

c. With a 25 ml graduate, transfer 15 ml of the appropriate solvent into each beaker. Stir with a 3 inch glass rod until the liquid phase is completely dissolved.

d. Through a glass funnel, transfer each liquid phase solution into one 300 ml Morton flask. Note: From this point on, all solvent used for rinsing beaker(s) and funnel(s) will be measured so that the final solvent volume in the flask will be just sufficient to produce a slurry of about heavy cream consistency when the support is added. This is a somewhat critical point in that too little solvent does not permit adequate mixing for uniform support coating, and too much solvent involves an excessive evaporation time for the solvent. A 10 ml Mohr pipet works nicely for adding and measuring the applied solvent. The beaker(s) should be rinsed with four consecutive applications of 7-9 ml of solvent, the exact amount depending on the appropriate solvent/support ratio.

e. After the liquid phase transfer into the flask, place a powder funnel in the flask and add the support. Note: The amount of support to weigh out for a 21 gram batch is the difference, in grams, between the total amount of liquid phase weighed and 21 grams. For example, with a 21 gram batch of packing of 4% SE-30/6% QF-1:

SE-30 -----------.040 x 21.0 = 0.84 grams

QF-1 ------------.060 x 21.0 = 1.3 grams

2.1 grams

Total liquid phase:

21.0 - 2.1 = 18.9 grams of support

f. Attach the flask to a rotary (Rinco) evaporator.

g. Mix *slowly* for 10 minutes at room temperature with just enough vacuum applied to hold the flask on the evaporator.

h. Advance the hot plate control sufficiently to raise the temperature of water in the beaker to 45°C in ca. 20 minutes. Increase the vacuum slightly at the start of heating and continue increasing, a little at a time. *Notes:* (a) By the time the temperature reaches 45°C, the vacuum should be such that the slurry is at a near-boil. This condition should be maintained throughout, until all visible solvent is removed. (b) After the 10 minute initial mixing period, the flask is rotated *very slowly*. This is a *very critical point*. It is generally not possible to slow the power stat or Variac sufficiently to completely accomplish this, and it is necessary to brake further by hand. This requires *continuous* attention by the operator throughout, really a small time investment in light of the importance of good column packing and the length of time good columns should give service.

i. Advance heat gradually to 55°C applying as much vacuum as possible just short of flushing liquid solvent out of the flask. Remove all visible solvent at this temperature.

j. Advance heat to produce 65°C applying all vacuum available and rotating *very slowly* and *intermittently*.

k. When all evident solvent is removed, release the vacuum carefully and shut down the assembly. Transfer the flask of packing to an oven and hold at 130°C at least two hours, or overnight.

Alternative pan coating and filtration coating procedures are described in the FDA PAM, Section 301.5.

Once a column is prepared, the actual weight percent loading can be determined, if required, by exhaustive Soxhlet extraction in glass thimbles or standard low temperature or thermal ashing procedures.

4H PACKING THE COLUMN

Columns for pesticide analysis are generally 4-7 feet (120-210 cm) in length and 1/8 or 1/4 inch (0.32 or 0.64 cm) od metal or glass. Aluminum columns have been found suitable for chlorinated pesticides, but glass is usually preferred to prevent degradation often associated with metal columns. U-shaped, 6-foot, glass columns are used in the Tracor MT-200 gas chromatograph which is standard throughout the EPA network of laboratories (Section 5). These are cleaned before packing by scrubbing with soap and water and a pipe cleaner, rinsing with water and acetone or anhydrous methanol and drawing vacuum to dry. Glass columns can be silanized prior to packing for chromatography of especially labile compounds, but this has not been found necessary for routine work.

There are several methods for packing a column, e.g., hand vibration, mechanical vibration, and vacuum. The method of choice may be dictated by the configuration of the column. Thus, vacuum is about the only method for packing a coiled column. A U-shaped column may be packed by any of the three methods. In general, the aim is to pack the coated support tightly to increase efficiency, with the least amount of particle breakage possible to decrease adsorption/degradation problems. The recommended method is hand vibrating, which has produced columns of consistently high quality.

a. The operator should be sure that the column, if intended as a 6-foot column, is really 6 feet in total length, and not some lesser length. Efficiency and retention time are both reduced in a shorter column. For off-column injection in some chromatographs such as the MT-220, one end of the column should be 1 inch shorter than the other.

b. On each column leg place a mark at a point on the glass that will be just visible at the Swagelok nut when the column is installed in the oven.

c. Through a glass funnel attached to the column, pour ca. 6 inches of packing into each leg.

d. Repeatedly tap the U-bend of the column on the floor for ca. 30 seconds. Note: The glass is fragile and it is therefore advisable to place some type of padding such as a magazine on the floor.

e. Repeat this operation, adding ca. 6 inches at a time to each column leg. It is advisable to vibrate additionally with a wooden pencil, running it up and down the length of the packing.

f. Continue adding packing and vibrating until the pencil marks are reached and the packing will not vibrate below the marks. This should be done with great care, tapping the column a sufficient length of time to be certain that no further settling is possible by manual vibration. The use of mechanical vibration is not advised as the packing may be packed too densely, thus introducing the possibility of excessive pressure drop when carrier gas is applied.

g. Place plugs of ca. 1 inch length of silanized glass wool in each end, just tightly enough to prevent dislodging when carrier gas is applied but not so tight as to impede gas sweep through the column. If glass wool is packed by hand, the hands should be carefully washed with soap or detergent, rinsed, and dried to minimize skin oil contamination of the glass wool. Glass wool can be silanized by treating with 10 percent dimethyldichlorosilane in toluene for 10 minutes followed by rinsing with toluene and treating for an additional 10 minutes with anhydrous methanol and air drying, or the prepared material can be purchased commercially (e.g., from Applied Science Laboratories). The column is now ready for conditioning.

Straight metal columns are plugged at one end with glass wool. The column is filled in portions with a minimum vibration and then the plugged end is tapped on the floor to settle the packing material. The other end is plugged after filling, and the column is formed into the required shape. Coiled glass columns are packed by plugging one end with glass wool and applying slight vacuum to this end to suck the packing material in increments into the tube. The tube is vibrated and tapped throughout to obtain a firm, uniform packing.

Stainless steel screens with 5 or 10 μ openings (available from Supelco, Inc.) have been recommended as replacements for glass wool in both GC and LC columns to improve column efficiency and protect gas sampling valves, if used, from column debris. Their use has not been evaluated for pesticide analysis to our knowledge, but it is good practice not to allow pesticides to contact hot metal surfaces during chromatography.

One excellent measure of a well packed column is the net weight of packing per foot compared to a previous efficient column. Experienced chromatographers can repeatedly prepare columns within ca. 2 mg/ft using the same batch of packing.

4I COLUMN CONDITIONING

The column is conditioned, or made ready for routine use, by heat curing, silylation or Carbowax treatment, and injection of a concentrated pesticide solution.

Heat curing of some EPA (Subsection 5L) and FDA prescribed GC columns is carried out according to the following schedule in order to eliminate liquid phase bleed, thereby improving the day-to-day stability of sensitivity and baselines, quantitation, and the quality of chromatograms, and lowering the amount of detector cleaning needed:

Phase	Oven Temp., °C 1/	Minimum Time, hr.
4% SE-30/6% OV-210	245	72
1.5% OV-17/1.95% QF-1	245	48
3% DEGS 2/	235	20 3/
10% OV-210	245	48
10% DC-200 4/	250	16
10% DC-200/15% QF-1 (1:1)	250	72-120
15% QF-1/5% DC-710 (2:1)	240	120

1/ Carrier gas flow 60 to 70 ml per minute.
2/ Shown for information only. Column not recommended for routine use.
3/ Do not exceed this time period.
4/ DC-200 columns are significantly improved if conditioning is carried out without carrier gas (11).

In general, it is desirable to heat cure the column at a temperature ca. 50°C higher than its operating temperature with a flow of carrier gas passing through the column in the normal direction. Details for the connection of the inlet column leg (which is 1 inch shorter for off-column injection) to the inlet port of a MT-220 chromatograph through a special Swagelok attachment are given in the EPA PAM Section 4,A,(2),IV,1. The column exit is vented inside the oven and not connected to the detector. The outlet ports leading to the transfer line are sealed off with Swagelok nuts to prevent traces of column effluent from seeping through to the detector. Particular caution is needed when preparing mixed columns with different, but supposedly equivalent, liquid phases. Use of one or more of the newer, stabilized liquids may give a column with an altered phase ratio after conditioning because of increased temperature stability. Although these columns are more stable, conditioning before use is still required.

As mentioned previously, column efficiency and response, especially the response of endrin, would slowly improve as new columns become "seasoned" with use, but silylation is a means of rapidly conditioning the column to full endrin response. After heat curing and with the column still isolated from the detector, the oven temperature and carrier gas flow rate are adjusted to the approximate recommended operating conditions for the column of interest (Subsection 5L). Four consecutive injections of 25 µl each of Silyl 8 (Pierce Chemical Co.) are made, spaced 30 minutes apart. Following the final injection, about 3 hours is allowed for all traces of the silanizing material to elute from the column. The syringe used for these injections should be used for no other purpose and should be rinsed immediately after use to avoid damage. The effects of silylation do not persist indefinitely, and repeat treatment about once a month is recommended. Columns to be used with flame photometric or thermionic detectors for detection of organophosphorus pesticides should not be silylated but rather Carbowax treated.

Generally, Carbowax-treated columns are much more responsive and capable of higher peak resolutions for organophosphate pesticides than columns that are untreated. Depending on the specific compound and column, increases in response have ranged from 10 to 200 percent, with a 100 percent increase, or doubled response, being most likely. Silyl 8 conditioning has no beneficial effect on organophosphate response, and silylated columns should definitely not be used with the flame photometric detector since bleed will cause excessive fogging of the heat shield. Details of the treatment and a special Swagelok assembly used in the MT-220 chromatograph are given in Section 4,B,(2),IV of the EPA PAM. This is a modification of the method reported by Ives and Guiffrida to bleed from a two inch 10 percent precolumn heated in the chromatograph oven at 230-235°C for 17 hours with a carrier gas flow of 20 ml/minute.

The response characteristics of the column should be monitored with a standard mixture of organophosphorus pesticides immediately after treatment to serve as a reference point for later checks on the longevity of the beneficial effects. Response will sometimes drop rapidly for several days after treatment and then stabilize, usually at a level well above that for the untreated column. Carbowax treated glass wool may also be less adsorptive than the silylated wool usually used.

Following the silanizing injections or Carbowax treatment and with the oven temperature and carrier gas flow rate adjusted to the approximate operating levels for the particular column, several successive injections of a chlorinated pesticide priming mixture in the microgram range are made onto the column with enough time between injections for all compounds to elute. Injection of priming standards each morning will help assure consistent peak response for working standards throughout the day. With some easily degraded compounds such as underivatized monocrotophos, the column is primed before every analysis. Other difficult pesticides which may not chromatograph well unless the column is aged and primed include perthane, methoxychlor, dicofol, tetradifon, chlorobenzilate, Prolan, captan, esters of 2,4-D, malathion, azinphos-methyl, coumaphos, and PCP.

4J EVALUATION OF THE COLUMN

Unfortunately, many chromatographers, after packing and conditioning the column, proceed immediately to use it without making the effort to systematically determine whether it is good or bad. Considering the fact that the column, if properly prepared and maintained, may be in constant use for a year or more as the most vital component of the gas chromatograph, the two or three hours spent conducting a systematic evaluation is time well invested. In fact, learning immediately whether the quality characteristics are sufficiently good to justify placing the column on-line as a working tool could result in a considerable overall time saving.

Full details of the evaluation procedure are included in Section 4,A of the EPA PAM. The following material provides highlights of the procedure.

After completion of conditioning steps, the oven and carrier flow are shut down, and the column is connected to the detector. A clean glass injection insert and septum are also installed. The oven temperature and carrier flow are then raised to their operating values. When the proper oven temperature is reached, the carrier flow rate is carefully tested with a soap bubble device and adjusted. (Subsections 5A and 5B discuss the proper performance of temperature and flow rate measurements). At least one hour, or preferably overnight, is allowed for the chromatograph to equilibrate. The temperature and flow rate are rechecked after equilibration. Before making any injections, a background (standing) current profile is run at the normal operating parameters for the specific column being tested if an electron capture detector is used. The polarizing voltage is set at its proper value. These operations are further discussed in Subsection 5C of this Manual.

A complex chlorinated pesticide mixture is now chromatographed to evaluate efficiency, resolution, compound stability, and response characteristics. The mixture described in Section III, C,5 of the EPA PAM is useful for this purpose since it contains compounds which give a number of very closely eluting peaks on the recommended pesticide GC columns. If the mixture is prepared in isooctane and stored tightly stoppered in the deep freeze, it is useable for a year or more for column evaluation (but not quantitation).

From the chromatogram of this mixture one can calculate the column efficiency based on the peak from p,p'-DDT. For successful pesticide analyses, this should be at least 500 plates per foot, or 3,000 plates for a 183 cm (6-foot) column, as calculated from the equation shown in Figure 4-C. The relative retention time for p,p'-DDT will indicate the actual column temperature (Subsection 5A of this Manual and Section 4,A of the EPA PAM) and serve as a check on the instrument pyrometer readout.

The absolute retention time of the p,p'-DDT peak should be 15 to 18 minutes or the operating parameters are incorrect, the column is not the correct length, or it is not properly packed. Too low an absolute retention indicates too high an oven temperature or carrier gas flow, too short a column, packing which is too loose, or a combination of two or more of these. A high retention time would indicate the possibility of opposite causes.

If column efficiency and resolution are favorable, compound breakdown is evaluated by injection of p,p'-DDT and endrin. Columns indicating poor resolution, efficiency, and/or retention characteristics which cannot be corrected by slight parameter adjustments should not be further used. On the other hand, satisfactory columns will often improve or "season" with use, especially as cleaned-up sample extracts are injected onto the column. The percentage composition of the liquid phase undoubtedly changes with age for most columns as well.

Pure analytical standard p,p'-DDT and endrin are injected in turn in sufficient concentration to result in a total peak height of 50-60% full scale recorder deflection. Breakdown, as indicated by appearance of peaks in addition to the main pesticide peaks, should not exceed 3 percent for DDT and 6 percent for endrin of the amounts injected. The breakdown percentage is the value of all peaks on each chromatogram divided into the total peak area value for the breakdown peaks x 100.

Carbamate pesticides are especially difficult to chromatograph directly on any current GC column without breakdown or tailing.

Reproducibility of the size of peaks when a compound is injected repetitively should be $\lessapprox$2-3 percent. Poor reproducibility can be due to breakdown or adsorption of the compound on the column or to extra-column causes such as faulty syringe or syringe technique (Section 5J), a leaky septum (Section 5J), or detector malfunction. Reproducibility should be checked with those compounds that are possible to chromatograph successfully but that can break down or be adsorbed (e.g., endrin). Priming injections of large amounts of a difficult compound, as mentioned earlier, may allow maintenance of reproducibility for an adequate period of time for an analysis. Difficult compounds should also be checked for linearity of response (Section 50d) since one cause of non-linearity may be on-column breakdown or adsorption.

4K MAINTENANCE AND USE OF GC COLUMNS

Table 3-3 of Section 3 outlines a recommended maintenance program for a gas chromatograph with an electron capture detector in monitoring laboratories in which biological media are predominate samples. A properly cared for column should provide service for many months. Off-column injection of biological samples will enhance column life (Subsection 5J); frequent (daily) changing of the injection insert and septum helps insure continuing good performance. Weekly, bi-weekly, or monthly, depending on the number and types of samples injected, the silanized glass wool plug at the column inlet should be replaced. This is mandatory when injecting biological samples directly on-column. If the glass wool plug becomes contaminated by extraneous material, chromatograms showing excessive DDT breakdown, peak tailing, and depressed peak height response will result. Changing the glass wool regularly will usually restore proper performance.

The column packing near the inlet must also be replaced with fresh, conditioned packing if it becomes contaminated. Contaminated packing can be removed without removing the column from the instrument by applying gentle suction through a long-tipped disposable pipet inserted into the column. The column interior should be swabbed where the packing was removed to eliminate fatty deposits on the glass wall. Elimination of the glass wool at the column inlet has been recommended (FDA PAM, Section 301.9) for minimizing fatty extract buildup at the top of the column by permitting the extract to spread over the top portion of adsorbent. This adsorbent, which will trap or degrade pesticides less readily than contaminated glass wool, is regularly replaced. Daily monitoring of DDT breakdown is important for early indication of contamination of the injection port and/or column. Improved cleanup of dirty extracts prior to gas chromatography is an obvious aid in maintaining good column performance.

The effects of silylation conditioning do not last indefinitely, and breakdown of endrin should be monitored weekly to determine if and when the treatment must be repeated. The effects of Carbowax treatment appear to persist for at least three months under normal use. The operator should watch for a slow decrease in the response of organophosphorus pesticides as compared to that produced by the column immediately after the initial conditioning. A repeat Carbowax treatment of the same column appears to rejuvenate the response, but may cause a shift in some retention values relative to parathion. Repeat treatments are therefore not recommended since consistent relative retention values are important for tentative peak identification (Subsection 5N).

When the column is idle overnight or weekends, a low carrier flow of ca. 25 ml/minute is maintained through the column and a simultaneous purge flow of 25-30 ml through the detector. When an instrument has multiple columns connected to a single EC detector, a carrier flow just high enough to provide positive pressure is maintained through the unused column(s). In a series of observations with a pair of nearly identical lowload columns having the same 70 ml/minute flow through each, the peak height response for aldrin was reduced ca. 25 percent compared to when the off-column had a very low carrier flow. If the column not in use is of a highly stable liquid phase such as OV-1, OV-17, etc., the carrier flow on this "off" column may be reduced to zero with no ill effects, thus allowing for full response from the column in use.

Columns removed from an instrument are tightly capped and are reconditioned if out of the instrument for more than a few days. A flow of 60 ml/minute carrier gas for several hours at a temperature ca. 25°C above the prescribed operating temperature (venting into the oven) is used for this operation.

Erratic and noisy baselines frequently indicate leaks in the column connections or some other point in the flow system between the injection port and the detector inlet. If the chromatograph oven can accommodate two or more columns but only one is installed, the unused transfer line to the detector must, of course, be plugged to prevent a massive leak.

Further details of instrument maintenance, troubleshooting, and calibration are given in Section 9.

a. Carrier Gas

Impure carrier gas can often virtually and irreversibly destroy a column. The main manifestations of this are evident in the inability to obtain an adequate background current profile, and low or zero response upon injection of standard solutions. Every effort should be made to avoid installing a new column for evaluation at the same time a new tank of gas is placed on-line. With this situation, the chromatographer cannot be sure whether he simply has a bad column or a bad tank of gas. If the problem is traced to a bad tank of gas, the molecular sieve filter at the inlet of the flow system should also be replaced as experience has indicated that the contamination of the molecular sieve will perpetuate the problem even after a fresh column and good tank of gas are installed (Subsection 5C).

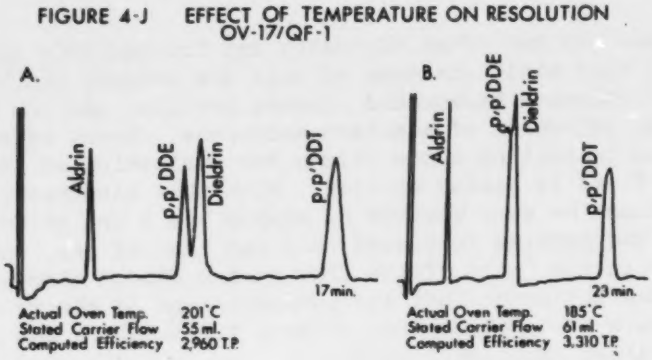
b. Erratic Baselines

This phenomenon may be caused by a number of instrumental factors and these will be treated in detail in Subsection 5K. The contribution of the column to this problem is largely one of loose joint connections, allowing air to seep into the carrier system. Special care should be taken to ensure that both column joint nuts are tight. One common occurrence is this: The chromatographer connects the freshly conditioned column to the detector and makes certain that the Swagelok nuts are tight. After about two days of operation, the oven door should be opened and the nuts should be tested with a wrench. In almost all cases, it will be found that the nuts are no longer tight, sometimes requiring as much as a half turn for retightening.

c. Accuracy of Oven Temperature and Carrier Gas Flow Velocity

Information gleaned from the interlaboratory check sample program described in Section 2 has clearly indicated that in many laboratories the chromatographer does not really know his true column temperature or carrier gas flow velocity. In most such cases, full reliance is being placed in the accuracy of the instrumental pyrometer and ball rotameter, both of which may be grossly inaccurate. These subjects will be discussed in Subsections 5A and 5B but are highlighted here because of the profound effects on the day-to-day operation of GC columns. Figure 4-J is presented as an illustration. A temperature of 200°C is recommended as optimum for the 1.5% OV-17/1.95% QF-1 column. At this temperature the separation between *p,p'*-DDE and dieldrin is normally as shown in Chromatogram A. One laboratory reported operation at 200°C but their chromatogram was that shown in B. Subsequent investigation revealed that the actual oven temperature was 185°C, or 15°C at variance with the value given by the instrument pyrometer. Resolution or quantitation of either *p,p'*-DDE or dieldrin would not be possible in Chromatogram B.

Figure 4-J. Effect of temperature on resolution, 1.5% OV-17/1.95% QF-1 column.



d. Sources of Supply of Blank Glass Columns

This subject is mentioned here only by reason of a very significant variation in prices between various suppliers for the same commodity. Price markups in excess of 700 percent are not uncommon, so it behooves the laboratory purchasing group to do a little shopping to achieve the appreciable savings possible on quantity lots.

The cited subsections of Section 5 treat these problems in greater detail as they relate to overall operation of the gas chromatograph.

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OPERATION OF THE GAS CHROMATOGRAPH

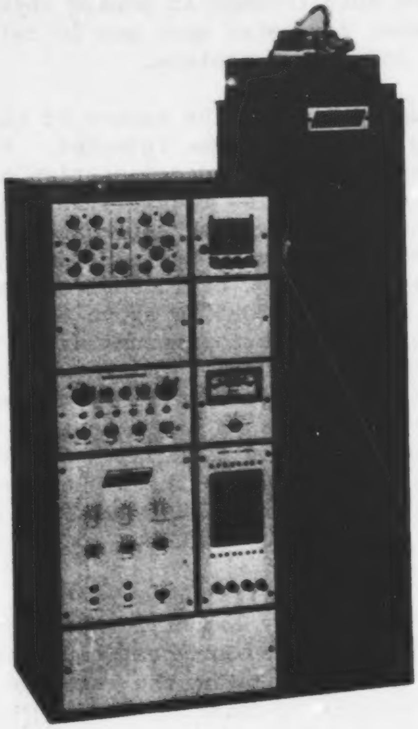
During the extended period of operation of the interlaboratory check sample program described in Section 2, a significant number of analytical "bloopers" have been attributable to improper operation of the gas chromatograph. In many such cases the operator had no idea that anything was wrong, primarily because no systematic guidelines were followed for monitoring the instrumental performance. This section will present such guidelines for the proper operation of the gas chromatograph in pesticide residue analysis. Some of the material is repetitive of the instructions outlined in the EPA Pesticide Analytical Manual, but because of its importance in analytical quality control, it was considered worthy of reemphasis. Section 4 should be consulted for material on evaluation, standardization, and maintenance of GC columns and Section 9 for details of instrumental troubleshooting and calibration.

Since the gas chromatograph is the instrument in most widespread use in the pesticide residue laboratory, its proper maintenance and use is of primary importance. Failure of any of the components, such as the oven, gas flow system, detector, electrometer, or recorder, to function at optimal potential can markedly distort the overall instrument performance and the resulting qualitative and quantitative data. Table 3-3 in Section 3 outlines a series of periodic checks recommended for insuring a continuing high level of chromatograph performance. Figure 5-A at the top of the next page shows the MT-220 (Tracor, Inc.) gas chromatograph which is in widespread use throughout EPA laboratories. This is a floor model chromatograph which features four vertical U-columns, on or off column injection, and simultaneous installation of up to four different detectors.

5A TEMPERATURE SELECTION AND CONTROL

Proper adjustment of the column oven temperature and the carrier gas flow rate (Subsection 5B) will have a great influence on the caliber of performance of the entire chromatographic system. Improper selection and control of these parameters may result in poor column efficiency with concurrently poor resolution of peaks, inaccurate relative retention values, depressed peak height response (poor sensitivity), elution times that are either too fast to yield adequate peak resolution and reliable peak identification or too slow to be practical, or rising or erratic baselines. Impaired resolution may preclude accurate quantitation of two important pesticides which are not adequately separated, while inaccurate retention values will make proper residue identification difficult.

Figure 5-A. Gas Chromatograph, Tracor, MT-220



The temperature regulation and readout systems of the column oven, detector, and injection port of the gas chromatograph are critical for obtaining reliable analytical results. Accuracy of the pyrometer readouts must be established and maintained to prevent occurrences such as electron capture detector tritium foil vaporization due to excessive temperature or an injection port or column significantly higher or lower in temperature than desired.

A properly operating temperature programmer will maintain the column oven temperature without appreciable deviation (±0.1°C), provided that room temperature fluctuations are minimal. Excessive temperature fluctuation will lead to erratic baselines and retention measurements. Pyrometer batteries should be checked monthly to determine if they are delivering full voltage under load. A hint of inaccurate pyrometer operation is obtained by switching to an unused sensor and observing the readout. A value more than 5°C from room temperature suggests faulty operation. In addition, the oven temperature must be monitored by means other than the built-in instrument pyrometer. A precalibrated dial thermometer with the stem inserted through the oven door or a mercury thermometer placed down through an unused injection port is recommended. The instrument pyrometer must not be relied upon as the only means of monitoring column temperature.

Injector temperature is determined by the nature of the sample, the identity of the pesticide, and the volume injected. An excessive injection port temperature may lead to decomposition of heat-labile pesticides, stripping of the partition liquid from the front end of the column resulting in peak tailing, and increased septum bleed (leading to spurious peaks) and reduced septum life. A temperature lower than the optimum may cause slow or incomplete sample volatization. The detector temperature should be 30-50°C above that of the column (50°C above the final temperature when programming is used) to prevent the possibility of condensation of sample components or liquid bleed from the column. An excessively high detector temperature can result in reduced sensitivity and/or increased noise level. Inaccurate column temperature can affect peak retention times and resolution and may alter the elution pattern of certain pesticidal compounds which may be present in a sample, sometimes to the extent that two compounds that completely separate at a given temperature may completely overlap at some other temperature.

Column temperature may be checked by computing the relative retention ratio for *p,p'*-DDT (or another convenient pesticide) compared to aldrin as follows: divide the distance in mm on the recorder chart between the injection point and the peak maximum for DDT by the distance between the injection point and the aldrin maximum on the same chromatogram. There is a linear relationship between column temperature and relative retention values so that comparison of this computed value with those available for over 50 pesticides on the recommended pesticide columns between 170°C and 204°C (EPA PAM, Subsection 4,A(6), Tables 2(a) - 2(c)) should provide a check of the actual column temperature. Selected values for *p,p'*-DDT are shown in Table 5-1 as an example. A computed relative retention value much below the given value in the table at the selected oven temperature indicates a temperature that is actually higher while a value much higher than the chart denotes a low oven temperature. Relative retention ratios are also a function of the type and proportion of the component liquid phases in the column packing, so preparation of the column and packing should also be carefully checked if retention values are discrepant.

Surveys of the data and chromatograms submitted by laboratories newly participating in the EPA Interlaboratory Control Program (Section 2) indicated that a high proportion of gas chromatographs were operating with column oven temperatures deviating significantly from the supposed values. These erroneous temperatures resulted from inaccurate instrument pyrometers and a lack of alternate temperature monitoring devices and procedures. As an example, one laboratory using a column of 1.5% OV-17/1.95% QF-1 indicated an absolute retention time of 26 minutes and a relative retention ratio of 4.87 for p,p'-DDT at a temperature of 200°C and flow rate of 65 ml/minute. Under these stated conditions the retention time for DDT should have been 18-20 minutes, and reference to Table 5-1 shows the true oven temperature was ca. 185°C, fifteen degrees less than the pyrometer indicated. See Figure 4-J in Section 4 for illustration of the effects of inaccurate column temperature on peak resolution.

A discussion of the importance of the GC oven to chromatographic performance and suggestions for simple evaluation techniques for thermal variables have been published (1).

TABLE 5-1

RETENTION TIMES FOR *p,p'*-DDT RELATIVE TO ALDRIN

Liquid Phase	Temperatures, °C								
	170	174	178	182	186	190	194	198	202
1.5% OV-17/1.95% QF-1	5.57	5.39	5.20	5.01	4.83	4.64	4.46	4.27	4.09
4.0% SE-30/6.0% QF-1	4.04	3.92	3.80	3.67	3.54	3.43	3.30	3.18	3.05
5% OV-210	4.47	4.31	4.15	3.98	3.82	3.66	3.49	3.33	3.17

5B SELECTION AND CONTROL OF CARRIER GAS FLOW RATE

The exact carrier flow system depends on the chromatograph in use. A common arrangement is for the gas to flow from the cylinder through a two stage regulator to a filter-drier element, branching thereafter to: (1) a purge line running through the purge rotameter, flow controller, and detector, and (2) the carrier gas flow line running through the rotameters, the flow controllers, the column, and finally through the transfer line into the detector. The choice of carrier gas is dictated mainly by the requirements of the detector being employed. Nitrogen is required for the usual pesticide detectors, except that the pulsed mode of the electron capture detector usually employs argon with 5 percent methane. Flame detectors require gases such as hydrogen, oxygen, and air for combustion.

Gases should be obtained in the highest possible purity and gas cylinders equipped with dual stage regulators. "Prepurified" nitrogen is required for the DC mode and, in some cases, the constant current pulsed mode of electron capture detection. A gas that is 99.998 percent pure has an impurity level of 20 ppm, and at least this purity should be employed for the carrier and auxiliary gases in trace analyses. Each gas supply is filtered through a filter-drier cartridge connected at the regulator output of the cylinder. A filter containing Linde 13X (1/16 inch) molecular sieve pellets will remove water, most hydrocarbons, and CO_2. Before the filter-drier is charged with the fresh molecular sieve, the interior of the cartridge is acetone rinsed and heated at 130°C in an oven for at least one hour. The bronze frit is acetone rinsed and flamed. After filling, the unit is heated at 350°C for four hours with a nitrogen flow of ca. 90 ml/minute passing through the unit. If activated units are to be stored for a period of time, the ends must be tightly capped. The filter unit should be replaced with a fresh one in the rare event one discovers that a contaminated tank of gas has been used. Oxygen removal requires a special scrubber or a molecular sieve filter immersed in liquid nitrogen. Gas cylinders should always be replaced before they are completely empty.

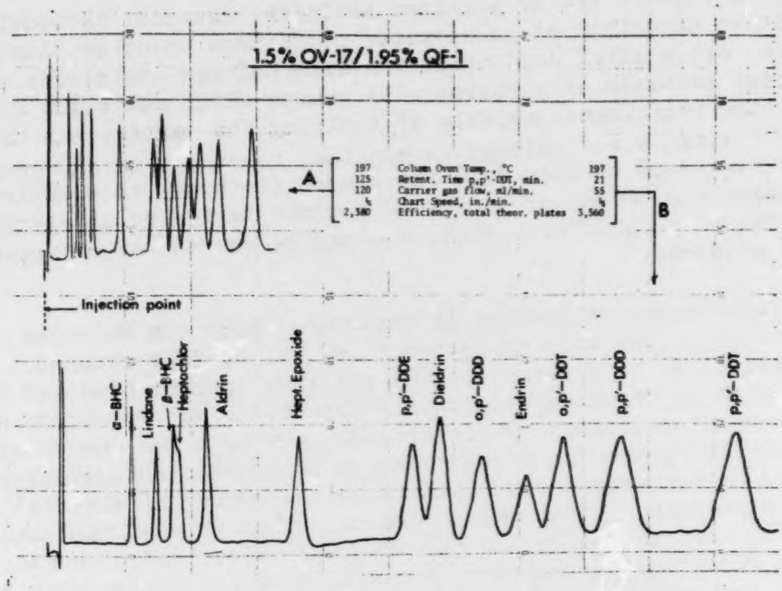
It is essential that no leaks exist anywhere in the flow system. Even a minute leak will result in erratic baselines with the ^{3}H or ^{63}Ni electron capture detectors. If the baseline has been stable but becomes erratic upon installation of a new column, a loose column connection is indicated. Leaks are detected by application of "Snoop" or some similar product at all connections in the flow system from the injection port to the detector, provided the connections are at room temperature. Do not attempt to use "Snoop" on a hot column. Another means of detecting leaks when using an electron capture detector is by spraying connections with Freon MS-180 with the instrument operating and observing any recorder response. Short sprays are applied close to the connection, but not around the injection port or the detector.

The carrier gas flow velocities are checked using a soap bubble flowmeter, which can be purchased commercially or easily constructed by attaching a sidearm near the bottom of a 50 ml buret (Subsection 4,A,(6), Figure 4(a), EPA PAM). Since rotameters are installed ahead of the columns, they cannot be relied upon when adjusting the carrier flow as they may be in error. It is necessary to check the flow rate at a point after the column because the pressure drop across columns will vary somewhat from one column to another. An equilibration period of only a few minutes with normal operating parameters is required before the flow rate check is made.

If two or more columns are connected to the same detector via a common transfer line, the carrier flow to the column(s) not in use is shut off while the flow rate through the column in use is being measured. Likewise, the purge gas is shut off. If flow in all columns is shut off, the purge gas flow through the detector can be measured. The flow through unused columns is also shut off while determining the background current of an electron capture detector. The head pressure gauge on some commercial instruments allows continuous monitoring for problems upstream of the column, such as a leak in the carrier gas lines, as well as determination of minimum regulator pressure, changes in column head pressure during temperature programming, and long term column changes. If available, head pressure monitoring can accomplish some of the same results as checking of the flow rate.

Carrier flow rates in excess of recommended values lead to lowered absolute retention times and compressed chromatograms while rates that are too low will have the opposite effect. Relative retention values reflect only the operating temperature of the column (Subsection 5A) while absolute retentions indicate either or both carrier gas flow rate and temperature. Other effects of excessive flow rate may include depression in peak height response and poor column efficiency. Figure 5-B illustrates chromatograms of identical pesticide mixtures from the same OV-17/QF-1 GC column operated with approximately the recommended conditions (B) and then with too rapid a flow rate (A).

Figure 5-B. Effect of Flow Rate on GC Resolution



GC Detectors

GC detectors for residue analysis must be sensitive to minute amounts of the pesticides sought, but selective enough not to detect reasonable amounts of co-extracted substrate material. Despite this selectivity, it is necessary to protect the total gas chromatographic system, including the detector, by purifying the extracts. This will reduce the amount of co-extracted material in the final solution to a level that will not be detrimental to the chromatograph or to the quality of the separation, identification, and measurement. Nonselective detectors, such as the flame ionization detector, produce very complex chromatograms with peaks from pesticides as well as from co-extracted compounds; these detectors are not selective enough to be practical for the quantitative analysis of residues of only a limited number of pesticides of certain classes. With detectors that are less selective (or less specific) for pesticide(s) of interest, more effort is required in sample preparation, in avoiding reagent contamination, and in residue identification.

The reader is directed to references (2-5) for general reviews of the element selective pesticide detectors.

5C ELECTRON CAPTURE

a. Principles and Operation

The electron capture (EC or electron affinity) detector is widely used for sensitive detection of halogenated pesticides or other classes of pesticides, often after derivatization with halogen-containing reagents. The detector consists of a radioactive source which emits low energy β-particles (electrons) capable of ionizing the carrier gas to produce secondary electrons. A voltage is applied, causing a steady stream of these secondary electrons to flow from the source (cathode) to a collector (anode) where the amount of generated current is fed to an electrometer and recorded on a recorder. Thus, a standing current or background signal is produced.

When an electronegative species is introduced into the detector, a quantity of electrons will be captured and the current reduced. The negative signal is in contrast to the positive current produced in detectors such as the flame ionization detector. The magnitude of standing current reduction, which depends upon both the number of electron capturing species present and on their electronegativity, is measured on the recorder and indicates the amount of material capturing electrons. After the component passes through the detector, the standing current returns to the original value, and a characteristic GC peak is shown on the recorder, provided that the radioactive foil is not overly contaminated. The exact theory of operation of the EC detector is still unresolved (6-8).

The EC detector is selective in principle for highly electronegative compounds, but in practice it is the least selective of the widely used pesticide detectors. Rigorous cleanup of pesticide extracts is required to eliminate extraneous peaks due to compounds containing halogen, phosphorus, sulfur, nitrogen dioxide, lead, and some hydrocarbons. Its sensitivity, however, is the highest of any contemporary detector, with many halogenated compounds being detectable in low pg (10^{-12}g) amounts. */ Advantage is taken of this sensitivity by preparing halogenated derivatives of compounds (e.g., carbamate insecticides) normally not detected well by EC. The response of EC detectors has been studied and guidelines presented for predicting which derivatives might best increase sensitivity (9).

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*/ Sensitivity of a GC detector is designated as the amount of pesticide that will provide a peak whose height corresponds to some percentage of the full scale recorder deflection (usually 10 or 50 percent). Minimum detectable amount is that quantity of pesticide giving a signal at least four times the background noise (random fluctuations) at baseline.

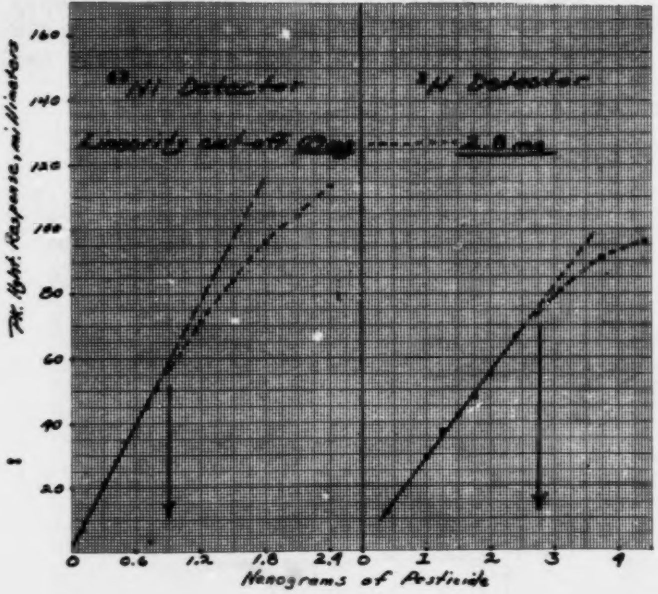
Sources of β-radiation have usually been either tritiated titanium on copper or a ^{63}Ni foil. The latter is more expensive but can be used at temperatures above 250°C, which would damage the tritiated detector (maximum temperature ca. 225°C). The nickel detector can be used safely to 400°C without appreciable loss of radioactive material. The higher operating temperature reduces the possibility of contaminating the detector with extract impurities or the bleeding of GC liquid phases. It also extends the number of compounds that can be detected and greatly reduces detector maintenance.

The EC detector is used with either a constant negative DC voltage or an intermittently pulsed voltage (constant frequency or "plain pulsed" mode) imposed across the anode-cathode. The former mode, which is most used, requires nitrogen carrier gas, while argon plus 5-10 percent methane is used with pulsed voltage. The argon-methane can be added to the chromatographic system as a make-up gas or as the carrier gas. When added as a make-up gas, introduced after the column but prior to the ionization portion of the detector cell, nitrogen or helium can be used as the carrier gas, and simultaneous dual detector operation is possible. The pulsed and DC modes provide approximately equal sensitivity and linearity, but advantages have been claimed for the former in terms of freedom from anomalous responses (10), reproducibility of response, independence of response to voltage, and when working with somewhat dirty samples. However, DC operation has proven entirely adequate for routine analyses in the EPA Laboratories when properly cleaned-up samples and low-bleed columns are employed.

Constant current (pulse- or frequency-modulated or variable frequency mode) operation is a third mode of EC detection. A standing current is again achieved by applying voltage pulses, but in this case the pulse sampling frequency is varied by a servo-mechanism closed loop control circuit that maintains the standing current constant even when an electron absorbing compound enters the detector. Pulse frequency is converted to a DC signal that is monitored in the usual way to provide a chromatogram. This mode of operation provides an increased linear response range without loss of detectability and a high degree of baseline stability, but it has not been carefully evaluated for routine pesticide work (11-13). Linearized ^{63}Ni constant current EC detectors are available from several commercial sources. They allow detection of low pg amounts of chlorinated insecticides with isothermal or temperature programmed operation and have a linear dynamic range of 10^4-10^5. This compound-independent, extended linearity is of great benefit for automated analyses where a wide concentration range of samples can be analyzed without dilutions or reruns. Those detectors with small cell volumes (ca. 0.3 ml) are well suited to capillary column GC. Most pulsed EC cells require argon-methane carrier gas, but at least one commercial version has been designated to permit efficient electron collection (0.1 pg detection of aldrin and heptachlor) with nitrogen carrier gas (14). This model functions as a concentration-type detector, as indicated by a dependence of linearity range on flow rate and retention time, and has sufficient baseline stability for temperature programming at the 10 pg level. A constant current tritiated scandium EC detector with high frequency pulsed operation (6) had an extended linear range to 10^6 and reduced nonlinearities compared to those previously reported (15) for lower frequency detectors with strongly electrophilic compounds such as aldrin. The tritium source is more sensitive than nickel for a short period of time, reaching maximum sensitivity after a few days of operation. Then there is typically a constant loss in sensitivity, requiring frequent recalibration and eventual foil replacement. The sensitivity of the ^{63}Ni cell is reputedly less than that of a tritium cell, but it remains relatively constant and may equal or surpass the sensitivity of a tritium cell after a period of use. Some compounds show increased sensitivity at the higher temperatures possible with the Ni cell than with a new tritium cell (10).

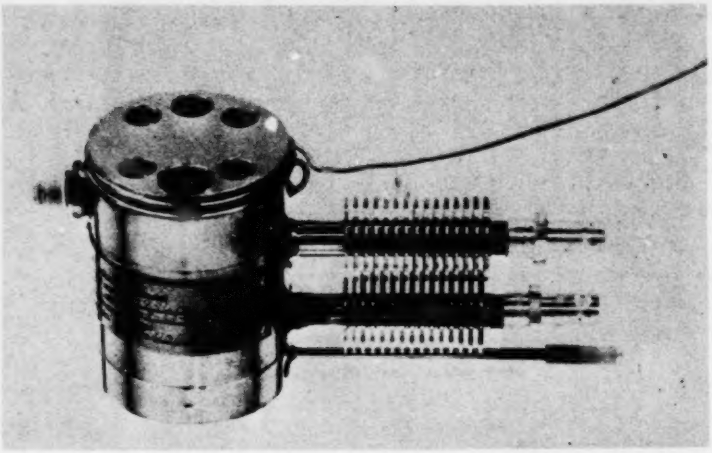
Traditionally, the linear range of the tritium detector has been three to five times greater than that of the DC ^{63}Ni detector. Figure 5-C shows typical linearity curves for p,p'-DDD using these detectors, both operated in the DC mode. When a certain point in concentration is reached, the linearity curve begins to "plateau". For quantitation, it is mandatory that concentrations of pesticide injected be within the linear or straight line range of the detector. Since this range may change with the age and use of the detector, standard curves must be determined regularly. With the Tracor MT-220 chromatograph, operation at an output attenuation of 10 x 8 or 16 on the E 2 electrometer or 10^2 x 8 or 16 on the SS electrometer will usually preclude violation of the linear range of the EC detector simply because peaks generated by excessive concentrations of pesticides will go off-scale. Samples should be diluted to give quantifiable peaks at these settings so that non-linear responses are avoided (see Subsection 5O on quantitation).

Figure 5-C. Comparison of linear ranges of ^{3}H and ^{63}Ni detectors for p,p'-DDD



The EPA analytical laboratories have mainly used parallel-plate ^{3}H EC detectors because cleaning can be done in-house under an NRC permit. Details are given in the FDA Pesticide Analytical Manual, Vol. I, Section 311.12, for cleaning a tritium EC detector. Foils may be removed only by persons with an NRC license for this purpose. A cleaning solution of 5 percent KOH in absolute ethanol is recommended for cleaning radioactive foils. Tritium foils should not be exposed for more than one hour, and aqueous solutions or traces of water should be avoided. Mildly abrasive cleaning compounds and ultrasonic cleaning apparatus may also be used. High temperature ^{63}Ni detectors are probably in greatest general use, and concentric-design tritium detectors are also used. Figure 5-D shows a Tracor, Inc. ^{63}Ni detector. The column effluent entrance is shown on the left and the purge gas line, polarizing voltage connector, electrometer input connector, and gas effluent outlet (top to bottom) on the right. The heater-limit switch fits into the nearest large hole seen on the top, front. The ^{63}Ni foil is a sealed source and is usually sent back to the manufacturer for cleaning. It is frequently possible, however, to clean a nickel foil in the chromatograph by injecting 100 µl of water a few times into a 300°C system employing an empty column. Purging the detector at 400°C overnight may also be helpful. It has been reported (6) that the major contamination of the EC detector occurs by deposition of material on the anode surface, causing a significant reduction in efficiency of collection of electrical charge, and that performance can be restored by cleaning only the anode without disturbing the other parts of the cell.

Figure 5-D. Tracor, Inc. ^{63}Ni EC Detector

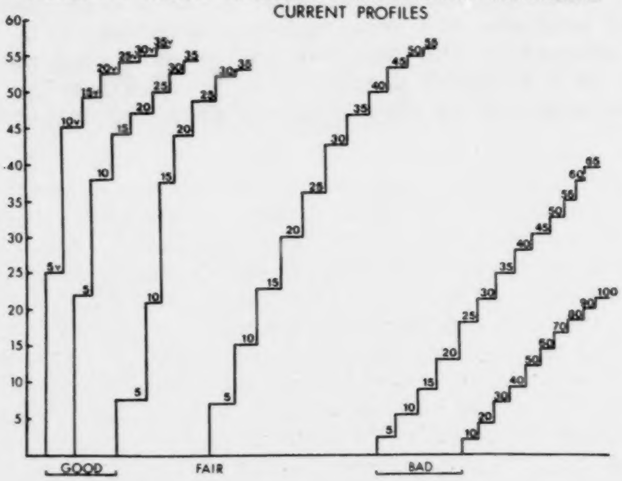


Response of EC detectors depends upon temperature (16); type, flow rate, and pressure (17) of the carrier gas; cell and electrode configuration and dimensions; electrode positions; amount of radioactivity; contact potentials caused by adsorption of sample components on electrode surfaces; space charges of slow moving ions surrounding the electrodes; and applied potential. The adverse effects of even slight scoring on the EC collector probe have been described (18). The unpredictable nature of these parameters causes anomalous responses, drifting baselines, variable sensitivity, and a limited, variable dynamic range in the DC mode. Operating parameters must be optimized for each manufacturer's detector. Electron capture detectors containing tritiated scandium (19) and ^{147}Pr gold foil (20) sources have been described. The former can be operated at 325°C and has reportedly three times the sensitivity of the usual tritium cell while the latter is heat stable and lower in cost than ^{63}Ni foils (10). These detectors have not been evaluated in day-to-day use in the EPA laboratories.

b. Background Current Profile

Measurement of the background current profile (recorder response vs. voltage) should be made regularly to evaluate the performance of the detector as influenced by the condition of the foil or other factors such as column bleed or contaminated carrier gas. At an attenuation setting of 12.8 x 10^{-9} A.F.S. when using a 1 mv recorder, a good detector should produce a response of 60-80 percent full scale deflection, and with aging this will approach 30 percent, when the foil should be replaced. A profile which drops drastically in a period of one or a few days indicates problems with the detector itself or an adverse influence by the column. Detailed instructions are given in Section 4,A,(3) of the EPA PAM for obtaining a BGC profile with a Tracor MT-220 chromatograph, and typical profiles are illustrated in Figure 5-E. Operational details for obtaining background current vary somewhat from one instrument to another, and each particular instrument manual should be consulted for recommended column parameters for making this test. Some commercial detectors regrettably do not provide for easy variation and readout of the potential. In general, more significant information is obtained by determining the background current at the normal operating parameters for the column being used.

FIGURE 5-E. TYPICAL ELECTRON CAPTURE DETECTOR BACKGROUND CURRENT PROFILES



From the background current profile, the optimum polarizing voltage may be estimated. If the detector foil is new and the background current is high, it is usually acceptable practice to simply set the polarizing voltage at such a value to give 85 percent of the total profile with the ^{3}H detector or at 92 percent with the ^{63}Ni detector.

A more reliable method, especially for older, partially dirty detectors, is to run a polarizing voltage/response curve as described in Section 4,A,(3) of the EPA PAM. Selection of the proper polarizing voltage is very important so as to (1) produce maximum peak height (response) with minimum electrical overshoot on the backside of the peak (Figure 5-S,A), and (2) ensure maximum possible efficiency and peak resolution. The polarizing voltage must be adjusted to accommodate a slowly deteriorating background current, so frequent profiles must be run to keep a check on this. An article published in Gas-Chrom® Newsletter March/April 1973 treated this subject so well, a reprint is presented on the following two pages, courtesy of Applied Science Laboratories, State College, PA.

Performance curves for pulsed operation are obtained by increasing the applied pulse voltage while maintaining a constant pulse rate and width. Rather than the typical stepped curves for DC operation (Figure 5-E), the detector response (recorder deflection) rises smoothly to a maximum at a relatively low pulse voltage (e.g., 25 V) and then continues at a nearly constant response plateau with higher voltages. An operating voltage is chosen well above that necessary to just give maximum response (i.e., 50-55 V) in order to allow a considerable margin for response reduction as the detector is used with contaminating sample extracts. To evaluate the progressive contamination of a detector in pulsed-mode operation, the decrease in the peak response level for a given amount of a standard pesticide at a certain pulse voltage is monitored and compared to that obtained when the detector was first installed.

Chromatographer, Beware of Thy Detector!

We all know that the performance of the same types of GC columns can vary with the quality of the packings and the columns themselves. Figures 1 and 2 are examples of extreme differences in column performance when used for pesticide analysis. Both columns are 6 ft x 4 mm ID glass U-tubes packed with 10 wt % DC-200 on a silane treated support. Both runs were made at the same operating conditions. Resolution in Figure 1 is good, but the resolution in Figure 2 is far superior because of the unbelievable 13,000 theoretical plates obtained. Yet, the separation factors are the same in both cases (e.g., 1.12 for endrin/dieldrin).

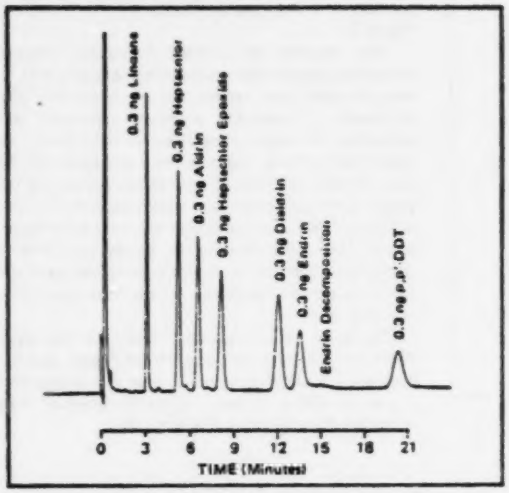


Figure 1. Chromatogram of standard chlorinated pesticide mixture. Column: 6 ft x 4 mm ID glass packed with 10% DC 200 on a silane-treated support. Column temperature: 200°C. Detector: Electron capture at 1 x 10^{-9} AFS.

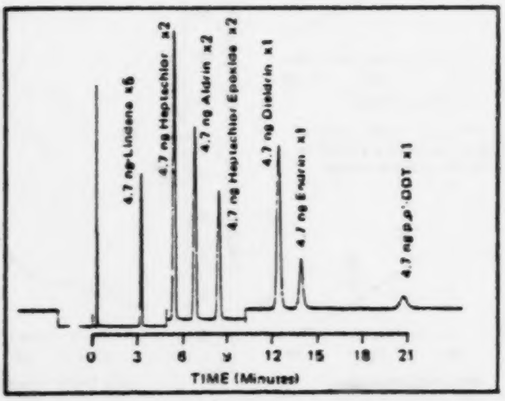


Figure 2. Chromatogram of standard chlorinated pesticide mixture. Column: 6 ft x 4 mm ID glass packed with 10% DC 200 on a silane-treated support. Column temperature: 200°C. Detector: Electron capture at 1 x 10^{-9} AFS.

You say that you would like a 6 ft column with the efficiency shown in Figure 2? Well, we can make such columns and we can obtain results like those in Figure 2 anytime we want to – providing we use an EC detector. In fact, we make these columns all the time, but do not obtain the Figure 2 results – because we do not want to. You ask why? A very good question.

Let's compare Figures 1 and 2 more closely. There is a noticeable difference in absolute detector response between the two results and also in relative responses among the peaks. In Figure 1, the endrin/dieldrin peak height ratio is good (0.61), with signs of slight endrin decomposition, which is normal. However, in Figure 2 the endrin/dieldrin ratio is only 0.31, indicating appreciably greater endrin decomposition, and yet there are no signs of it in the chromatogram. Something appears to be radically wrong with the results in Figure 2.

Well, something is wrong. The column used in Figure 2 does not produce 13,000 theoretical plates. Actually, it is the exact same column which gave the Figure 1 results. Everything was the same for the two runs except for one thing – the EC detector voltages were different. A voltage of 10 volts produced Figure 1, while a voltage of 30 volts produced Figure 2. The detector is an old Barber-Colman tritium EC detector with variable DC voltage; i.e., any voltage can be applied. A plot of background current (baseline) vs. detector voltage is shown in Figure 3. The problem is non-linear detector response. EC detector response is linear or approaches linearity only over a small range of voltages. This voltage range usually lies below the knee leading to the plateau in the current vs. voltage curve (see Figure 3). In our examples, this voltage range is approximately 10 to 15 volts.

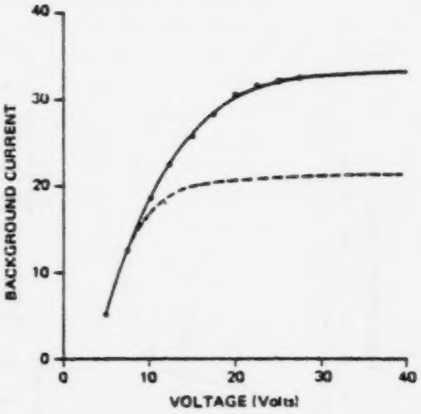


Figure 3. Plot of current vs. voltage for an EC detector.

At higher voltages, the response becomes non-linear and the response-to-concentration slope increases with increasing concentration. This non-linearity becomes extreme on the plateau of the current vs. voltage curve. Here, the response to concentration slope is very small at low concentrations and increases rapidly at high concentrations. This results in an extreme contraction of the lower part of a GC peak and an extension of the upper part of the peak. When this occurs, a chromatogram like the one in Figure 2 is produced. Figure 1 can be converted to an approximation of Figure 2, as shown in Figure 4. A superficial baseline has been drawn which cuts out the bottom part of the peaks. The similarity between Figures 2 and 4 is obvious. If we extended the upper part of the peaks in Figure 4, the chromatogram would resemble that in Figure 2 still more closely. This may appear extreme, but notice that in Figure 2 we have lost not only the endrin decomposition product, but also all the small impurity peaks that are seen in Figure 1.

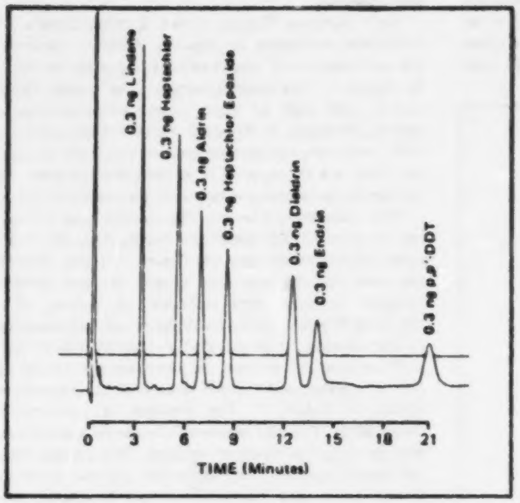


Figure 4. Same chromatogram as in Figure 1.

At voltages below the optimum range, the reverse occurs. The response to concentration slope is high at low concentrations and decreases with increasing concentration. In this case, the lower part of a peak will be extended and the upper part contracted. This is observed as a wider peak, giving a low theoretical plate calculation, and the peak maximum will tend to be rounded. Also, small peaks will be overemphasized.

The graphs in Figures 5 to 8 summarize the effect of EC detector voltage on GC results. These figures show the effect of voltage on various peak height ratios and on the theoretical plate calculation.

Another complication is that the current vs. voltage curve and the optimum voltage are not always the same, but vary with factors such as detector cleanliness and liquid phase bleed. A dirty detector or a high liquid phase bleed will cause the plateau in the current vs. voltage curve to shift to lower currents and voltages, as shown by the broken line curve in Figure 3.

This problem of variable non-linear response with EC detectors complicates quantitative analysis and is the reason why frequent and careful use of calibration standards is so important in pesticide analyses. However, when one is interested in determining column efficiency, the effect of non-linear detector response on the theoretical plate response has not been so obvious. This effect can also be observed with argon ionization detectors, where applied voltage also affects linearity. Years ago we found we were consistently obtaining about 150 more theoretical plates per foot from argon ionization detectors at voltages above the optimum than from flame ionization detectors, which have good linear response characteristics.

To be fair to EC detectors, there are now one or more on the market which operate at a fixed voltage and are claimed to have good linear characteristics over a wide dynamic range.

Let it suffice to say: "Chromatographer, beware of thy detector! Also, know thy detector!"

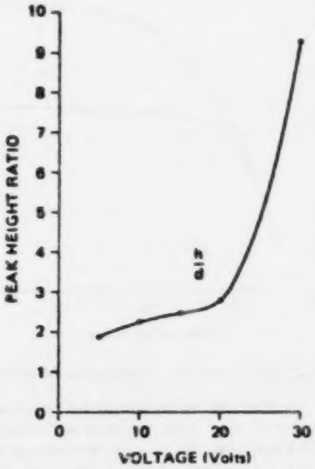


Figure 5. Plot of peak height ratio of heptachlor to dieldrin (h/d) vs. EC detector voltage.

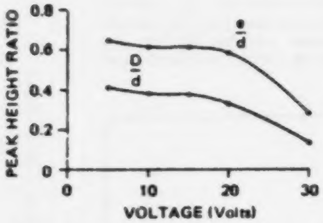


Figure 6. Plot of peak height ratios of endrin to dieldrin (e/d) and p,p'-DDT to dieldrin (D/d) vs. EC detector voltage.

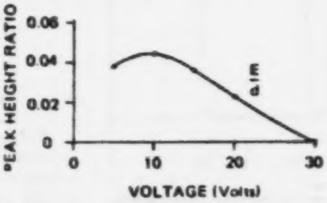


Figure 7. Plot of peak height ratio of endrin decomposition product to dieldrin (E/d) vs. EC detector voltage.

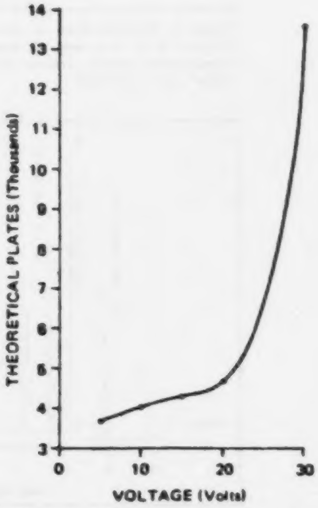


Figure 8. Plot of calculated theoretical plates for dieldrin vs. EC detector voltage.

c. Detector Contamination

Contamination of the detector by deposition of a coating of low vapor pressure materials on the electrodes seriously affects detector performance by consuming a portion of the detector capability, leading to loss of sensitivity, trailing peaks, or erratic chromatographic baselines. Contaminant sources may be a bleeding column, contaminated carrier gas, bleeding septum, dirty sample inlet, unclean samples, dirty carrier gas flow controller, or dirty tubing. Many of these factors are treated in detail elsewhere in this Manual, but a review is presented below.

Oxygen is a frequent contaminant in nitrogen carrier gas, and the EC detector responds exceptionally well to traces of oxygen. A background profile should be made after changing the tank of carrier gas and allowing at least one hour for the system to equilibrate. A suitable oxygen scavenger and a clean chromatographic system are most important for good performance (12, 21).

Certain liquid phases tend to bleed in varying degrees at normal operating conditions, even after conditioning for extended periods of time. DC-200, DC-550, and QF-1 are such phases and should be avoided where possible. The high temperature OV silicones with low (1-5 percent) phase loadings produce very favorable columns. The background current determination is particularly important with a new column in the instrument because background current that cannot be brought up to the expected level indicates the probability of a bleeding column requiring additional heat treatment to vaporize off the volatile impurities.

Solvents and monomers can bleed from the septum and be swept through the column into the detector. Glass inlet liners used for off-column injection should be changed frequently. Proper handling of septums and maintenance of the injection port are discussed in Subsection 5J

Contamination from dirty tubing or other system components prior to the inlet can be caused by a bad tank of carrier gas containing grease, oils, or water vapor. Use of a molecular sieve filter-drier will usually prevent this problem. These adsorbent traps must be regenerated regularly. If moisture has accumulated in the tubing and flow controllers from a bad tank of gas, simply changing the tank may not solve the problem. The entire system would have to be flushed out with a low boiling solvent.

A good rule is to introduce only one variable at a time into the GC-EC system and to run a background profile just before and just after changing the variable. For example, a column and a tank of gas should not both be changed at the same time. This rule makes the isolation and correction of problems a much easier task.

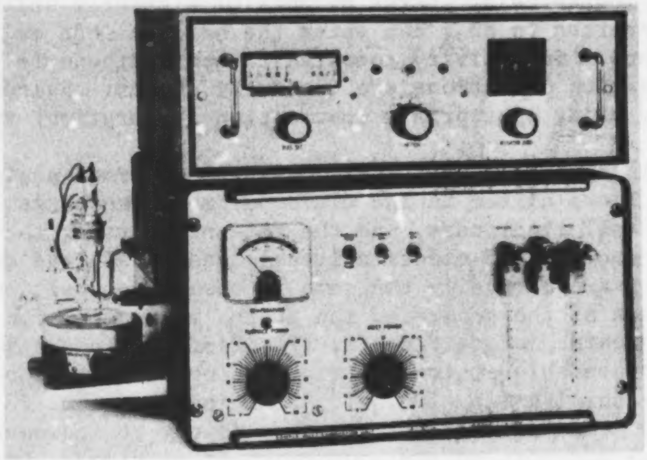
A review of the operation and principles of the electron capture detector for pesticide analysis (22) and a review of its theory and characteristics (10, 23) have been published.

5D MICROCOULOMETRIC DETECTOR

The original detector for organochlorine pesticides was the microcoulometric detector, but it has now been largely replaced by the electron capture detector and/or the electrolytic conductivity detector because of their greater sensitivity and easier operation and maintenance. Whereas the EC detector responds to all electron-capturing species, the MC detector can be operated to be specific for halogenated pesticides (or for pesticides containing S, P, or N). The latest modified MC detector has detection limits for halogen, sulfur, or nitrogen of 1-3 ng. The sensitivity for pesticides depends upon the percentage of these elements in the pesticide. With this relatively low absolute sensitivity, it is necessary to concentrate sample extracts to a greater extent and/or to inject larger sample volumes compared with the electron capture detector. Injection volumes of 50-100 µl are common with the MC system, and this volume may represent 10-25 grams of original sample. Cleanup procedures must accommodate the larger samples, but because of the increased detector specificity, cleanup often does not have to be as rigorous as for EC detection.

The MC detector, pictured in Figure 5-F on the following page, operates in the following manner. The pesticide is pyrolyzed in a furnace and the reaction product automatically titrated with an electrochemically generated reagent in the coulometric cell. The amount of ions generated can be related theoretically by Coulomb's Law to the absolute amounts of pesticides passing through the GC column, and peak areas can therefore be directly converted to a quantitative value by use of an equation, without need for a calibration curve.

Figure 5-F. Dohrmann-Envirotech Microcoulometric Detector



The various modes of detector operation are as follows:

Pesticide Class	Products of Furnace Reaction	Cell Reaction	Generation Reaction
Halogenated (Cl^-, Br^-, or I^-)	HX (oxidative, reductive, or catalytic)	$Ag^+ + X^- \longrightarrow \underline{AgX}$ (T-200-S cell)	$Ag^o \longrightarrow Ag^+ + e^-$
Sulfur	SO_2 (oxidative)	$I_3^- + SO_2 \longrightarrow SO_3 + 3I^-$ (T-300-P cell)	$3I^- \longrightarrow I_3^- + 2e^-$
Nitrogen	NH_3 (catalytic)	$NH_3 + H^+ \longrightarrow NH_4$ (T-400-H cell)	$H_2 \longrightarrow 2H^+ + 2e^-$

The coulometer and furnace systems require careful optimization to obtain accurate and precise results. Selectivity depends on the specificity of the preliminary and coulometric reactions. For example, any combustion products in addition to SO_2 (such as HX or nitrogen oxides) which can reduce iodine titrant will give a response in the sulfur mode with the iodine cell. Large amounts of elemental sulfur, not completely converted to SO_2, can enter the halogen mode cell and react with silver ions. Selectivity compared to hydrocarbons is good in all modes when reaction conditions are carefully chosen. Selectivity may be improved by using appropriate subtraction (absorption) tubes.

Titration cells have two operational positions, termed position I and position II. These differ in the microcoulometer settings, position of the titration cell electrodes, and the rate of stirring the electrolyte. The chemical reaction and electrical responses within the cell and microcoulometer are basically the same in these two positions; the rate of reaction of the generated ion at the surface of the sensor electrode is greater in position II. Position I is operationally simple and provides dependable stoichiometric (coulometric) response but is relatively insensitive (ca. 100 ng). Position II is ca. 20 times more sensitive (less than 10 ng), but response is not stoichiometric (calibration is needed) and more careful operation is required. Different models of the cell and coulometer and individual detectors of the same model will vary in sensitivity.

The MC detector operates over a wide temperature range, and its sensitivity is not affected by carrier gas flow rate, column bleed, or temperature changes. The detector is, therefore, satisfactory for use with temperature programming under special conditions (24). This is not generally recommended in pesticide analysis, however, because of poor precision of retention times from run to run. Peaks produced by the MC detector tend to be nonsymmetrical and tailing, leading generally to response that is not strictly linear in position II. In position I, constant recovery, which is the critical parameter rather than conventional linearity because of the application of Faraday's Law, is achieved above 500 ng of chlorinated pesticides.

In the period between 1960 and 1966, the MC detector was extensively used for pesticides containing Cl and S atoms, but since 1966 detection of nitrogen (e.g., carbofuran (25)) and chlorinated pesticides has predominated. The major use of the detector is for confirmation of halogenated pesticides in position II with the T-200-S cell. It is also advantageous for multicomponent chemicals such as PCBs because the chromatogram, unlike the response of the EC detector, will consist of peaks whose sizes are proportional to the amount of halogen present. The analysis of environmental residues of such compounds, the amounts of whose various components may change drastically from the original material being used for reference, is therefore facilitated. Use of the detector for phosphorus compounds has rarely been reported. The detector requires a relatively large amount of day-to-day maintenance and cleaning in order to keep it operating with sensitivity, selectivity, and stable baselines. Like the Coulson conductivity detector, manual venting of the solvent introduced during injection is required unless special automatic venting equipment (26) is installed. U.S.F.D.A. has published details of design and hints for successful MC detection of Cl and S (27).

5E ALKALI FLAME IONIZATION (THERMIONIC) DETECTOR

This detector was discovered about simultaneously by Giuffrida and Karmen in 1964. These workers showed that when the cathode of a flame ionization detector (FID) contained different alkali metal salts, the response for halogen- and especially phosphorus-containing pesticides was enhanced. Since then, improved response for compounds containing S, N, As, and B has also been noted. At least eight theories have been advanced for the phenomenon, but none completely explains the observed responses. Since response of the FID is directly related to thermally excited ions, the term "thermionic" has been used for the detector.

The original Giuffrida design consisted of a standard FID, the cathode of which was replaced with a Pt-Ir coiled wire coated with Na_2SO_4. An early commercial version had a salt tip made from a pressed pellet of CsBr added to the regular FID. The Cs tip enhances stability and working life compared to Na_2SO_4. Other salts such as KCl, KBr, Rb_2SO_4, and K_2SO_4 are also used. Response values for various compounds depend upon carrier and fuel gas flow rates, electrode geometry, distance from the flame, potential and polarization of the electrodes, carrier gas flow configuration, and the nature and purity of the salt. The type of metal and the hydrogen flow rate (flame temperature) seem especially important. Sensitivity and selectivity to particular elements is achieved by optimizing the above parameters. For example, phosphorus selectivity depends very sensitively on the fuel gas ratio, and alteration of geometry permits selective detection of compounds containing halogen and nitrogen at the expense of those containing phosphorus. Optimization can be time consuming, and frequent replacement of the salt tip is required if baseline stability, sensitivity, and quantitative response are to be maintained. Calibration should be performed with each analysis for reliable results. Large solvent injections disturb performance and should be avoided, and aromatic or halogenated solvents and acetonitrile are never used.

The greatest selectivity seems to be achievable for phosphorus, which can be detected about 1000-fold more sensitively than compounds containing N, S, or halogen. As an example, the KCl detector has an enhanced response for P of 10,000 or better (compared to the conventional FID), while the increase for N is 100 and As 30 (28). Other sources list selectivity ratios as high as 75,000/1 and 35,000/1 vs C for P and N, respectively (6). Selectivity for P with respect to hydrocarbons is usually reported as being poorer than that of the flame photometric detector, but detection limits are commonly stated as being potentially at least one order of magnitude better than for the FPD. Discrimination among N, S, and halogen compounds is generally quite poor.

Although the alkali metals Na, K, Rb, and Cs all increase selectivity for P markedly, the greatest sensitivity is produced by use of a potassium salt. For detection of nitrogen, the best combination of sensitivity and stability is obtained when a rubidium salt is used. Halogen response can be depressed by KCl and KBr, but sodium sulfate, carbonate, or nitrate increase response to Cl and Br compounds. Response to S and halogen compounds can be negative (inverted) but still related to the amount of compound present. The negative response has been used to detect chlorinated pesticides in soil at levels between 0.01 and 10 ppm without extensive cleanup of an exhaustive hexane extract (29). The amount of hydrogen in the flame must be optimized for each particular salt.

Sensitivities for organophosphorus pesticides are generally in the low ng range (e.g., 0.2-2 ng parathion for 1/2 fsd (28)), compounds with short retention times and narrow peaks being more sensitively detected. Reports of the detector's linear range have varied from 3-5 decades (13) to 0.5 to 20 ng (28). Minimum detection of triazine herbicides (N mode, Rb_2SO_4 tip) was reported as 0.2-0.5 ng with a 920 selectivity vs carbon compounds but a poor selectivity vs P (30). A 9:1 RbCl:RbBr coil gave about 1/2 scale deflection for 10 ng of various triazines (28). A detector with a KCl-Rb_2SO_4 (1:1) tip was used to determine ca. 30 ng of carbamate herbicides (31). Triazines, which contain a greater percentage of nitrogen per molecule than carbamates, would be expected to show a proportionately greater response.

In order to maintain the highest possible sensitivity of detection for N or P compounds and to avoid anomalies, such as spurious peaks, detector contamination must be minimized. The use of cyano-substituted silicone liquid phases (such as OV-225, OV-275, XE-60, and the SP-2300 series), H_3PO_4-treated supports and glass wool, phosphate-containing detergents, and Snoop leak detector should be avoided. Rapid deterioration of sensitivity will result from the loss of the alkali salt, most commonly caused by overheating the collector due to a major disturbance in gas flows (especially carrier and air). Care should be taken to turn off the collector voltage while changing the septum, columns, gas cylinders, etc. Cleaning with Freon (as is often done with the standard FID) will adversely affect the alkali salt of the thermionic detector (32).

A Varian Aerograph Rb_2SO_4 detector modified to allow increased carrier gas flow up to 60 ml/minute and injection volumes up to 7 µl has been described in detail (33). With this detector, greater than 1/2 fsd was achieved upon injection of 0.2-0.5 ng of six common organophosphorus insecticides. Details for preparation and operation of a KCl thermionic detector providing 1/2 fsd for two ng of parathion have also been given (28). The stability and sensitivity of a RbCl nitrogen thermionic detector were carefully compared with those of a conventional FID for the pesticide fenitrothion (34). A Pye 3-electrode RbCl thermionic detector has been tuned to give higher detection sensitivity for sulfur (positive detector response) compared to nitrogen but less than that for phosphorus. The relationship between hydrogen flow rate and optimum detection of the three elements was shown (35). The sulfur response of this detector, optimized in terms of electrode assembly position and flame conditions, was found to be more linear and as sensitive as the S-mode of the FPD, but selectivity against P was not as good (36). A KCl detector with changeable geometric arrangement of the source and the polarization electrode to vary the selectivity factor between P and N was described (37).

A new design of the thermionic detector provides increased stability by replacing the normal FID ion-collection assembly with a collector that has a ceramic cylinder coated with an alkali salt. The cylinder, centered about 1.25 cm above the flame jet, is heated electrically to a dull red color while a negative polarizing voltage is applied to the species collector. The ion current generated by the thermionic emission in the presence of P and N is measured with a conventional FID electrometer. The flame in this detector is not ignited. It is adjusted to give a low temperature plasma used for production and dissociation of ions from the organic compounds but not to heat the collector. The linear dynamic range is $>10^4$ for both N and P compounds, selectivity $>10^4$ grams C/gram N or P, and minimum detectable level $<1 \times 10^{-13}$ gram N/second and $<5 \times 10^{-14}$ gram P/second with H_2-He (8:92) support gas. The detector has been tested with folpet, dyrene, and atrazine (38). An earlier modified design of the thermionic detector utilizes a small, nonvolatile rubidium silicate glass bead between the flame and collector electrode. The bead is heated electrically and is, therefore, independent of the flame as a source of thermal energy. This detector, operable in phosphorus or nitrogen plus phosphorus modes, was preliminarily tested using standard malathion (39) and further studied (40) for optimization of the nitrogen response. The main advantage of this model is the ability to partially compensate for aging of alkali volatilization by continuously increasing the heating current. The latest version of this detector (41) has rapid, automatic changeover among three modes (normal FID, P, P + N), performed by operating push-botton electrical switches and pneumatic valves on a control unit. Neither of these detectors has been evaluated for routine pesticide analyses. A still earlier flameless detector for P pesticides, termed a "chemi-ionization" detector, utilized an electrically heated CsBr atmosphere. It was found less sensitive than the flame thermionic detector but quite selective (42).

In summary, compared to the FPD and electrolytic conductivity detector for detecting P- and N-containing pesticides, the flame thermionic detector is generally the cheapest, most sensitive, least selective, but least rugged (12). Its optimum, reproducible use is more of an art than science, and it is recommended mainly for experienced gas chromatographers. Reviews of the thermionic detector have been published (43, 44).

5F FLAME PHOTOMETRIC DETECTOR (FPD)

This detector operates by monitoring HPO and S_2 emission bands, which result from burning the column effluent in a cool, hydrogen-rich flame, at 526 nm (P-mode) or 394 nm (S-mode) using a combination of a narrow band-pass interference filter and a suitable photomultiplier tube. Samples require relatively little cleanup because of the selectivity of the detector for pesticides containing P or S. Applications to the detection of certain other elements (e.g., Ti, As, Zr, B, Cr) have also been made with limits of ca. 10^{-7} - 10^{-11} grams.

Figure 5-G shows the external appearance of the FPD. The carrier gas exit line is seen on top and the O_2/air inlet connection and hole for the heater on the bottom side. The mirror lies behind the circular bulkhead seen covering the burner chamber on the front, and the filter lies behind the screw seen on top of the photomultiplier housing. The hydrogen gas inlet is on the opposite side, and column effluent enters underneath the burner chamber. Signal and polarizing cables are attached at the back end of the PM tube. A cross section view of the FPD is shown as Figure 5-H. Figure 5-I pictures the dual FPD which in principle allows simultaneous monitoring of sulfur and phosphorus output from a single injection, as well as normal flame ionization output if it is of interest. In practice, differences in sensitivity of the P and S modes make dual operation impractical for analysis of low amounts of residues where maximum sensitivity is sought, That is, if the P-mode is optimized, the S-mode will not be sensitive enough to be of use. Figure 5-A shows a Tracor FPD mounted to the MT-220 gas chromatograph. At least three other companies produce FPDs that differ in a number of construction aspects and performance.

Figure 5-G. Tracor flame photometric detector.

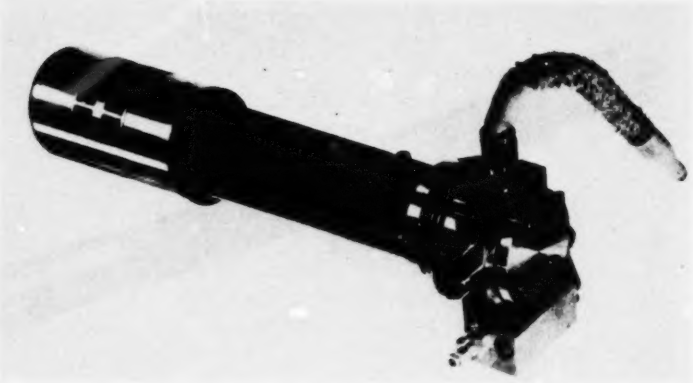


Figure 5-H. Cross section of a flame photometric detector

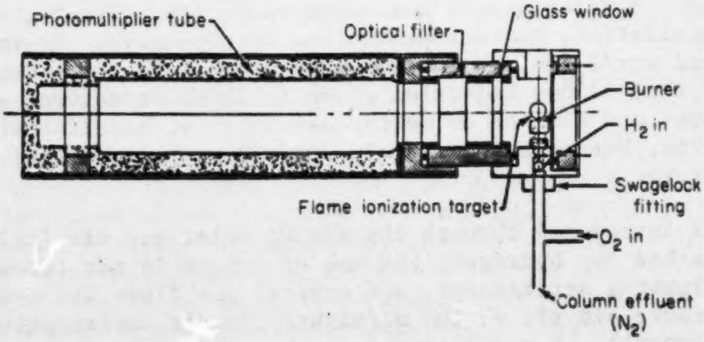
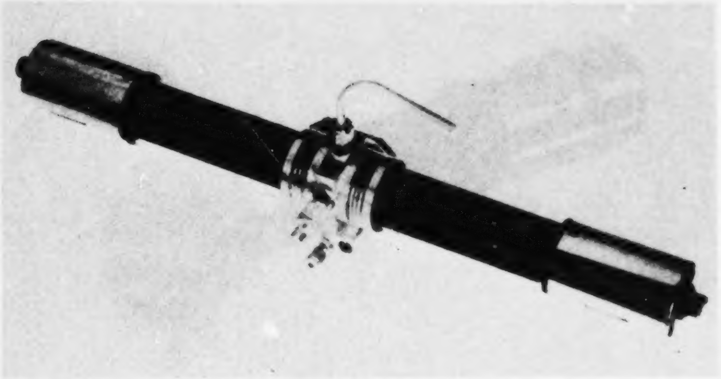

Photomultiplier tube
Optical filter
Glass window
Burner
H_2 in
Swagelock fitting
Flame ionization target
O_2 in
Column effluent (N_2)

Figure 5-I. Dual flame photometric detector



The original operating conditions for the FPD were as follows: oxygen (± air) is mixed with the nitrogen carrier gas and pesticide at the entrance to the detector; hydrogen is brought directly to the burner. Components are burned in a hollow tip which shields the flame from view by the PM tube. Emission occurs above the flame tip, and the light is transmitted to the PM tube through a filter which transmits a specific wavelength of the element to be monitored. A potential is applied to the PM tube and its output is amplified by the electrometer and read-out on a recorder.

When the FPD is operated in this manner, solvent in the injected sample will extinguish the flame unless modification is made to vent the solvent. A Valco 4-part switching valve (No. CV-8HT), silylated before installation, is recommended for this purpose. Reversal of the hydrogen and air/O_2 inlets has now been shown to give a "hyperventilated" flame (45) that allows injection of up to 50 µl of solvent with no flame blowout and similar or better sensitivity, baseline stability, and linearity, but an approximately 20-fold loss in selectivity for most detectors.

Hydrogen is introduced through the air/O_2 inlet and air (only) into the port marked for hydrogen; the use of oxygen is not recommended with this new plumbing arrangement, and typical gas flows are hydrogen 50-60 ml/minute and air 80 100 ml/minute (Tracor Instruments FPD Operation Manual). A new design dual-channel FPD with a separate combustion chamber, in which the sample is mixed with hydrogen before introduction to the detector, also resists flame-out with up to 50 µl of liquid sample (46).

The minimum detectable quantities of the elements S and P reported in different sources are about 40 pg-1 ng and 10-100 pg, respectively. In routine operation, 2.5 ng of ethyl parathion should yield a peak height equal to 1/2 fsd, although sensitivity can usually be improved well beyond this in most analyses by careful adjustment of operating parameters. A small addition of SO_2 to the hydrogen of the FPD flame caused an 8-fold increase in the sensitivity of the S-mode to aldicarb and malathion (47).

The degree of sensitivity of the FPD relates to four factors: condition of the PM tube; voltage applied to the PM tube; flow rates of H_2, O_2, and air; and the condition of the detector interior. Each of these factors should be checked and optimized for each installation. A drastic reduction in the peak height of malathion can be an indication of a poor column, provided the rest of the system is known to be operating properly. Equal amounts of malathion and ethyl parathion normally give a peak height response ratio of about 0.70 on a good column.

To obtain optimum flow rates, set hydrogen flow at 150-200 ml/minute, obtain maximum response for an injected, early eluting phosphate pesticide (e.g., ronnel or diazinon) by varying oxygen flow with zero air flow, then maximize response by varying the air flow with oxygen set at its optimum value. Some detectors may show best response with no air flow. Maximum response is indicated by a large signal to noise ratio; an increase in flow rates may increase peak height while also causing an increase in baseline noise. Typical operating parameters for FPD in the P-mode are in the table on page 29. (These values and most of the information in this section are for the Tracor FPD, used in most EPA laboratories, operated as originally designed. See the earlier paragraph on operating conditions when support gases are reversed and reference (48) for optimization of gas flow rates for the Pye FPD of different design.)

The temperature gradient between the column and detector is kept as low as possible, and the detector is always heated before the column when starting up a cold system. The detector base is maintained at about 210°C. The nitrogen to oxygen ratio should be ca. 4:1, the carrier and purge flow rates should be equal, and the total flow of air, oxygen, and carrier should not exceed 200 ml/minute. A lower total usually produces the most favorable signal to noise ratio.

Temperatures (^oC)		Flow Rates (ml/min.)	
Column	200	Purge*	70-80
Injection Block	210-225	Carrier	70-80
Detector**	165-250	Hydrogen	150-200
Transfer Line	235	Oxygen	10-30***
Switching Valve*	235-240	Air	0-100

* With Valco switching valve

** High temperature model is never heated above 250°C, low temperature model never above 170°C

*** To ignite the flame, an oxygen flow of 80 ml/minute or more may be required, depending on the detector

Although the FPD is not as sensitive to gas leaks as the electron capture detector, the flow system of the chromatograph should be tight. Leaks in the hydrogen, oxygen, or air supply can be hazardous from an explosion standpoint.

Optimum response voltage for the PM tube is determined using a variable power supply which allows the voltage to be increased with little increase in electronic noise. Raising the voltage from the electrometer will increase electronic noise inordinately. With optimum flow rates, the power supply is set at 750 V and a sample of diazinon injected to give 30-60% fsd. The injection is repeated at 850 V and at voltage increments between and around these values until the point of maximum signal to noise ratio is determined. It may be necessary to attenuate to keep on scale during this determination, so the linearity of the electrometer must be known. Different PM tubes require different voltages for best performance, a value of about 850 V being typical. A suspect PM tube may be checked with one of known sensitivity to give indication of its condition. Satisfactory operation of the FPD over its full dynamic range requires both a highly stabilized 750 V power supply plus an electrometer with a bucking capability of at least 1 x 10^{-6} amps. PM tubes are heat sensitive and should be well insulated from sources of heat.

Interference filters may be changed at any time, i.e., it is not necessary to shut down the instrument to do this. The power to the PM tube must be turned off when removing it from the flame base. Excess light will damage or destroy the sensing element when the tube is connected to the power supply. Light leaking into the PM tube during operation of the chromatograph will increase the noise level and decrease sensitivity. Ignition of the flame may be detected by observation of recorder pen deflection up or down scale, hearing the "pop" of the hydrogen gas, or deposition of moisture on a cold, flat metal surface held near the exhaust tube. The pyrex or quartz glass windows in the optical system can become etched through the action of alkali vapor and must be replaced or polished.

The FPD response to P is linear over a concentration range of about 3-5 decades, e.g., 0.4-400 ng for parathion in the P-mode. Nonlinear response of the FPD (526 nm filter) to oxygen analogs of OP pesticides is often noted and is thought to be caused by degradation of these $P = O$ compounds. GC columns should be optimized for separation of these compounds without breakdown, and metal transfer lines between the column and detector should be as short as possible and preferably made from Teflon or glass-lined metal tubing. The S-mode is inherently less sensitive than the P-mode, and response for compounds containing a single S atom is nonlinear starting in the 1-10 ng range. The response increases very roughly as the square of the concentration of sulfur, so standard curves are plotted on semi-log paper for S-mode quantitation. Quantitative evaluation of chromatographic data from the nonlinear S-mode FPD has been theoretically and experimentally studied (49). An option is available for commercial detectors that electronically transmits the square root of the detector response to the recorder so that plots of peak height or area vs concentration are linear within ± 5 percent. This linear response facilitates easy interpretation and allows electronic integration and data acquisition not possible without the square root function. The potential errors involved in the use of these commercial linearizers, if response is not actually proportional to the square of S concentration, have been evaluated and recommendations made to minimize the error (50).

In order to operate in the dual mode, it is necessary to optimize combustion gas flows for the S-mode and to have sufficient sulfur to detect in this mode. This combustion mixture is not necessarily the optimum for best phosphorus response. Optimum conditions will vary from detector to detector. If enough residue is present to detect in the S-mode, attenuation must be used to keep the P response on scale with the S response.

The proper attenuation for a given sample will depend upon the sensitivity achieved, but, in general, it is best to operate at the minimum detection level and to dilute the sample as necessary. Selectivities for P and S are about 10,000-25,000 or more:1 compared to nitrogen, carbon, hydrogen, and oxygen. Large amounts of sulfur impurities give a response in the P-mode (P:S response ratio 4-25:1 at 526 nm) whereas phosphorus impurities cause negligible response in the S-mode (S:P response ratio 100-1,000:1 at 394 nm). As the degree of sulfur oxidation in the molecule increases, there is usually a decrease in sulfur response. Factors affecting selectivity of the FPD have been studied (51).

Maximum utility of the FPD is afforded by the dual photomultiplier arrangement (Figure 5-I) whereby P and S are simultaneously monitored on a dual-pen recorder. This arrangement informs the analyst whether a compound contains only P or S, or both, and the P/S ratio (the P-response divided by the square root of the S-response) is important information for confirmation of residue identity. The response ratio ($x10^3$) ranges from 5.0-5.8 for PS compounds, 2.5-3.4 for PS_2 compounds, and 1.6-2.4 for PS_3 compounds (52). As mentioned earlier, dual operation will not be practical for analysis of low amounts of residue barely detectable by the P-mode of an FPD optimized for this mode because of the much lower sensitivity of the S-mode under these conditions.

Errors have been noted (53) in quantitations with the FPD in the P-mode when automatic integration is applied. The detector response passed through a minimum after the solvent peak and then gradually rose to the baseline without passing through a maximum to stop the integration before the first pesticide peak. This was overcome by adding a low boiling organophosphate (e.g., tributyl phosphate) that eluted after the solvent but before the pesticide peak (malathion was studied). The FPD has been coupled with a capillary GC column for analysis of OP pesticides (54).

The FPD has proven to be a versatile, sensitive, selective, and reliable means of analyzing not only pesticides and metabolites containing P and S atoms, but also for compounds such as carbamate insecticides in the form of derivatives containing these elements. The FPD appears to have advantages over the normal flame thermionic detector for routine analysis in terms of ease of operation, better stability, less maintenance time, independence of response to gas flow rates, and need for less frequent injection of standards. Sensitivity of the FPD may be about one order of magnitude less for P compounds than with a fully optimized thermionic detector. Applications and limitations of the FPD in atmospheric analysis have been reviewed (5).

Varian has developed a new FPD with dual flame design that is reportedly (55) superior, but it has not yet been carefully evaluated for routine residue analysis. Two hydrogen-air flames are used to separate the regions of sample decomposition and emission, so that the (second) emission flame is more efficient, and sensitivity is improved compared to the single-flame FPD. Besides reduced peak quenching, interference from hydrocarbon solvents is reduced because much of the C-H emission takes place in the first flame, which is not viewed by the photomultiplier. Reported selectivities are 10^5 grams C/gram P and 10^3 grams - 10^6 grams C/gram S, and response is less affected by the compound structure because of more complete breakdown into S_2 and HPO species. Up to 200 µl of solvent can be injected without extinguishing the flame, and a pushbutton linearizer for the exact quadratic response of the S-mode is included.

5G ELECTROLYTIC CONDUCTIVITY DETECTOR

a. Original Coulson Detector

This detector operates by combustion of pesticides eluting from the GC column to form simple molecular species that readily ionize and contribute to the conductivity of water deionized by passage through a mixed-bed ion-exchange resin column. Changes in conductivity are monitored with a DC bridge circuit and recorded on a strip chart recorder. The detector is pictured in Figure 5-J. Components include the pyrolyzer assembly with a separately heated vent valve, pyrolyzer furnace, heater and gas controls; the glass conductivity cell including the gas-liquid contactor; the water circulation cell consisting of water reservoir, water pump, and ion-exchange bed; and the conductivity bridge assembly. The conductivity cell is shown in Figure 5-K.

Figure 5-J. Tracor (Coulson) electrolytic conductivity detector

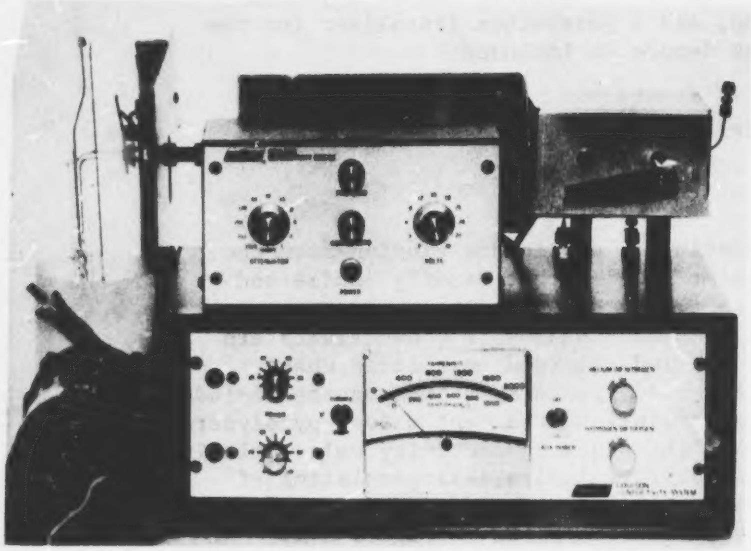


Figure 5-K. Electrolytic conductivity cell

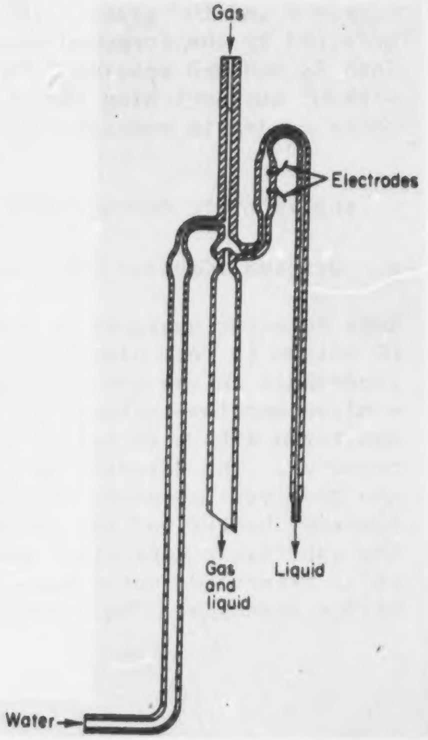


Table 5-2 shows the various modes of operation of the conductivity detector as described by Cochrane. Selectivity and sensitivity in these modes are governed by the furnace temperature, nature and flow rates of the reactant and carrier gases, flow rate of water through the cell, and proper choice of catalysts and scrubbers. Each analyst must optimize his conditions for the compound in which he is interested. As a result, the minimum sensitivity values reported by different workers for compound classes have varied quite widely.

TABLE 5-2

CONDUCTIVITY DETECTION MODES OF OPERATION

Mode	Conditions	Selectivity	Sensitivity
N	H_2 reduction gas - 850°C - Ni wire catalyst - NH_3 formed and detected as NH_4+ - He or Ar carrier gas	$Sr(OH)_2$ scrubber removes acidic products - responds only to N compounds	100 pg N; 100-200 ng subst. ureas, 7-15 ng triazines, 50-80 ng carbamates, 35-50 ng thiol-carbamates; 150 ng parathion, 25 ng diazinon.
Cl	empty tube reduction using H_2 gas - HCl produced - 600 or 850°C - N_2 carrier gas	responds to Cl and N-contg. OGP insect. such as diazinon	500 pg Cl; 6-40 ng Cl insecticides.
S	S-compounds oxidized to SO_2 and SO_3 in O_2 stream in empty tube - Cl converted to HCl - N_2 carrier gas - Pt gauze catalyst may be used - Furnace temperature 850°C	responds to S, Cl, and N - Ag wire scrubber increases selectivity with respect to Cl, but at expense of sensitivity and reproducibility - CaO scrubber for SO_2 has also been used	1 ng S; 30-2200 ng N compounds gave 1/2 fsd, depending on O_2 flow.
Pyrolytic	empty tube pyrolysis 400-600° or 850°C	responds to N, Cl, S	500 pg S and/or Cl, 1 ng N; linear range 2-1000 ng S; 6-100 ng S and Cl pesticides.

From: Cochrane, W. P., presented at May, 1973 Symposium on Pollutant Analysis, Athens, Ga.: See also J. Chromatogr., 75, 207 (1973); Int. J. Environ. Anal. Chem., 3, 199 (1974); and (59).

The best selectivity and sensitivity have generally been obtained for N compounds, and detection of these has been the major use of the detector up to now. A comprehensive study of 95 organonitrogen pesticides with 5 and 10 percent DC-200 columns gave a range of 1-1000 ng for 1/2 fsd (56). Sensitivities for several herbicides determined in foods (terbacil, propanil, linuron, DCNA, benzoprop-ethyl) ranged from 7-100 ng (1/2 fsd), and there were fewer extraneous peaks than with electron capture GC or HPLC (UV) (57). If the $Sr(OH)_2$ pledget and the catalyst are removed, the N-mode will detect organochlorine pesticides. Sensitivity for N compounds is better than with the microcoulometric detector, and similar to that of the rubidium thermionic detector. Selectivity for N compounds is reported to be superior to the thermionic detector (58). The S-mode of the conductivity detector is of comparable or somewhat better (59) sensitivity but less selective than the S-mode of the FPD. The conductivity detector is less complex than the MC detector and requires less maintenance, but reports differ on which requires cleaner extracts. The conductivity detector is much more selective but generally one or two orders of magnitude less sensitive than the electron capture detector, and analyses can be performed without cleanup using the former, whereas cleanup would be required for the latter (e.g., for determination of triazine herbicides (60, 61)). Heptafluorobutyryl and other perfluoro derivatives of pesticides were detected with a sensitivity of 1-5 ng in the halogen-mode, compared to 40-400 pg for EC detection. However, 1-2 ppb of residues in foods were determined by either detector because the greater selectivity of the conductivity detector permitted a greater quantity of sample to be injected (62).

Variables affecting the detection of nitrogen pesticides have been studied (63). Response was not affected by changes in gas flows from 10-100 ml/minute, while water flow rate through the conductivity cell of 1-2 ml/minute gave maximum response. Skewed chromatographic peaks and reduced sensitivity resulted from adsorption of NH_3 on contaminated quartz pyrolysis tubes or the pyrex glass detector assembly and even some brands of new quartz tubes. Cooling the deionized water through the detector increased sensitivity by reducing its background conductivity and background electronic noise. (A simple refrigeration unit has been designed to prevent temperature increases in the circulating deionized water and thereby increase uniformity of response throughout a day's operation of the detector (64)). Besides the GC and detector operating parameters, sensitivity for various compounds depended upon the number of nitrogen atoms and their molecular arrangement. As a specific example, the minimum response for methomyl oxime was 3 ng with linearity to ca. 38 ng with the following detector operating parameters:

Conductivity bridge - Voltage 30
- Attenuation 1
Pyrolysis furnace temperature - 800°C
Vent block temperature - 220°C
Gas flows - helium 80 ml/min.
- hydrogen 80 ml/min.

A study of the effect of operating parameters on the response of triazine herbicides (65) indicated maximum response with equal water flow rates in the mixing chamber and siphon arm. Tailing of peaks increased at slow water rates, and 1.2 ml/minute produced the highest response. Sensitivity increased with increasing furnace temperature, due to increased combustion of the pesticide.

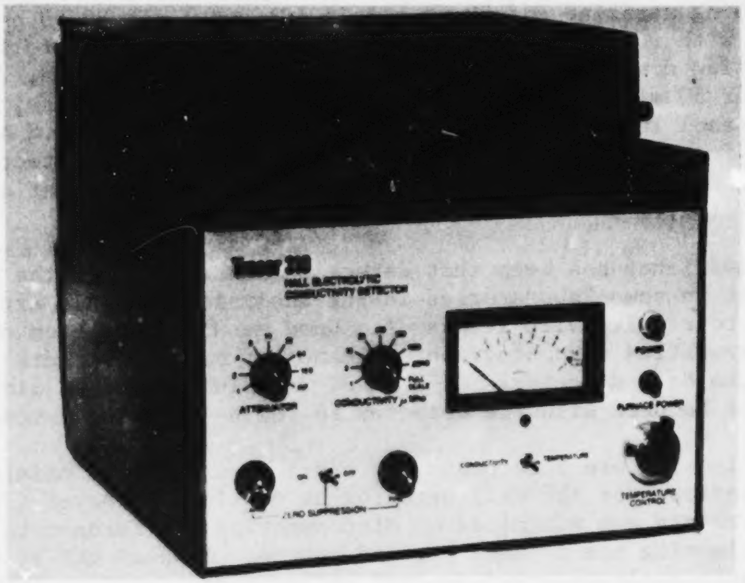
A general review of the electrolytic conductivity detector has been published (66).

b. Modified Conductivity Detectors

Using the reductive mode of operation, Hall (67) enhanced the selectivity and sensitivity of the conventional conductivity detector for organochlorine pesticides by modifying the reaction tube and furnace exit and increasing the maximum cell voltage. Heptachlor was detected by Hall at 0.1-0.4 ng levels compared to 2-5 ng for the original model, and chlorinated insecticides were detected with a 1000:1 selectivity over PCBs. Factors most affecting selectivity were furnace temperature and reaction gas composition and flow rate, while sensitivity was affected most by adsorptive surfaces, electrode polarization, system stability, and furnace temperature.

The commercial version of the Hall detector is shown in Figure 5-L. This detector features miniaturized one-package design, an improved furnace temperature controller, and an AC conductivity bridge. A low volume combustion tube and alcohol conductivity solvent instead of water eliminate need for solvent venting. (A vent valve has been described that reportedly eliminates solvent interference and increases reproducibility (68)). Response for Cl, N, and S is linear over a range of greater than 10^5, and peak broadening is greatly minimized. Selectivity vs carbon is 10^4-10^6 (69, 70). Chromatograms produced by Hall (71) illustrate the detection (15-90 percent fsd) of nine chlorinated insecticides at 0.9-6.5 ng levels with the chloride mode, and one ng simazine and 10 ng atrazine, diazinon, ethyl parathion, and carbofuran with the nitrogen mode (Ni catalyst, isopropanol-water solvent). The improved sensitivity of the Hall detector is seen by comparison of these values with Table 5-2.

Figure 5-L. Tracor Model 310 Hall electrolytic conductivity detector


Tracor 310
HALL ELECTROLYTIC
CONDUCTIVITY DETECTOR
ATTENUATOR
CONDUCTIVITY
FULL SCALE
POWER
FURNACE POWER
CONDUCTIVITY
TEMPERATURE
ON
OFF
ZERO SUPPRESSION
TEMPERATURE CONTROL

A further comparison of the Hall and Coulson detectors has been carried out. An approximate 7-fold increased sensitivity was found for the Hall detector relative to the Coulson detector for nitrogen-containing pesticides. Values obtained on a 4% OV-101/6% OV-210 column at 205°C were as follows (72):

	ng for 1/2 fsd	
	Coulson	Hall
Atrazine	7	1.1
Bladex	15	1.1
Chloropropham	75	6.0
Diazinon	25	4.5
Ramrod	50	6.5
Parathion	150	20

The Tracor company reports sensitivities of 40 ng of atrazine (N-mode) and 40 ng atrazine and 20 ng aldrin (Cl-mode) for 30-60 percent fsd peaks with <1 percent fsd background level under the following typical operating conditions: 6 feet x 1/4 inch 3% OV-1 column, 200°C, helium carrier 50 ml/minute, hydrogen reaction gas 50 ml/minute, 50 percent n-propanol in deionized water electrolyte flowing at 0.8 ml/minute, 850°C furnace temperature (73). Bayer reports easy detection of 0.5 ng of lindane in the reductive mode with an attenuation of 1 x 10 (32).

EPA experience has been that detection sensitivity of the Hall detector is lost in some laboratories in the analysis of sample extracts compared to results with standards. Good results have been obtained when gel permeation chromatographic cleanup of sample extracts is combined with the Hall detector. QF-1 or OV-210 fluorinated GC liquid phases may not be used with the detector in the N- or halogen-modes (74).

The following are some operating characteristics and maintenace instructions for the Hall detector as outlined by Bayer (32): Cleaning requirements are minimized by disconnecting the furnace to cell transfer line, leaving the furnace on, and turning the pump off at the end of the day's analyses. Build-up of carbonaceous residues in the quartz tube is alleviated by running the furnace at high temperature in the oxidative mode. Siliceous deposits resulting from silicon column bleed or silyl derivatives can be removed with 10 percent HF. Alternatively, the quartz tube can be replaced. Small variations in the conductivity solvent flow rate will change the detector response, so the flow rate should be set to a constant value each day. The recommended 1-5 cc/minute hydrogen flow rate through the standard 2 mm id quartz tube is very difficult to achieve in the reductive mode with the supplied needle valve, but variations have only minor effects on detector sensitivity. The maintenance and cleaning required depend on the type of samples analyzed. Weekly or more frequent cleaning may be required if dirty samples are commonly analyzed. The procedure, requiring less than one day, involves disassembling the unit and replacing the quartz tube, Teflon transfer line, ion-exchange resin, and solvent. The needle valve, its filter, and the conductivity cell are cleaned in an ultrasonic bath. Baseline noise can be caused by air bubbles or residue trapped in the conductivity cell and ionic species in the solvent. Bubbles are removed by rapidly turning the solvent pump off and on. Residues are removed by disassembling and cleaning the cell in an ultrasonic bath, and ionic species are minimized by using high purity solvents and water and routinely changing the ion-exchange resin.

A second modified detector was recently described by Lawrence and Moore (75) with a new, compact cell and optimized flow conditions and a water jacket for temperature control. A five-fold increase in sensitivity was achieved over the conventional detector with a water flow of 1.2 ml/minute and temperature less than 20°C. Two modifications were reported for this detector (76): An improved water flow control, consisting of a fine stainless steel wire inserted into the capillary tubing leading to the mixing chamber to reduce flow to the optimum value, provided 1/2 fsd for 2 ng of atrazine. Modified bridge circuitry allowing analyses to be carried out at 100 V increased the peak height for 5 ng of atrazine ca. 3 times compared to operation at 30 V.

5H OTHER DETECTORS AND DETECTOR COMBINATIONS

Other detectors have been employed on occasion for pesticide residue analysis, but none is as prominent as those covered in Subsection 5C through 5G. Bowman, Beroza, and co-workers (77-79) positioned a copper screen or pellet of Na_2SO_4 or In metal at the flame tip of a FID as in the thermionic detector. Compounds containing halogen modify the metallic atomic line spectral emissions which are monitored at characteristic wavelengths in a manner similar to the FPD. Detectability for aldrin was about 3 orders poorer than for the EC detector and selectivity with respect to hydrocarbons was 10,000:1 (inferior to the EC detector) and 100:1 to S- and P-compounds (better than EC). Response was linear with concentration on a log-log scale. Thorough extract cleanup was required with these detectors. The indium detector was combined with a conventional FPD to give a dual flame photometric detector for simultaneous and selective detection of P-, S-, and Cl-containing pesticides eluted from a GC column (80). In the lower flame, compounds emerging from the column are pyrolyzed and monitored for S and P. Between the two flames is a stainless steel net holding In pellets. The Cl-containing compounds interact with In and the resulting emission is monitored at 360 nm. Sensitivity for Cl is ca. 100 times less than the electron capture detector, linearity is 100 times greater, and selectivity is greater. The GC detection of halogenated pesticides based on the Beilstein response with Cu has been questioned because certain halogen-free organic compounds also release volatile Cu species in the flame and give a positive green flame (81).

The microwave plasma detector of Bache and Lisk is much like a FPD except that the relatively low energy flame is replaced by a much higher energy microwave plasma excitation. The plasma is established by irradiating an inert carrier gas such as Ar or He flowing through a tuned microwave cavity at either atmospheric or reduced pressure. All elements of organic compounds can be detected, and more than one element may be examined simultaneously. Detection limits for most elements are 0.1-1 ng/second (3 ng/second for N) with an adequate linear range. Organophosphorus pesticides, carbamates and triazines, and organomercurials have been determined with this detector (82).

The sulfur-phosphorus emission detector (SPED) is similar to the FPD except that fibre optic bundles are used to transmit light from the flame to the PM tube and that the chemiluminescence of the HPO and S_2 species are monitored at different heights above the flame (the viewing port for P is 6 mm above the port for S). This detector has been evaluated for pesticide analysis with the following results (59): response was similar to the normal FPD (linear in the P-mode, squared in the S-mode, and quadratic for compounds containing both P and S); linearity for three standards (Ro-neet, DEPPT, and DEPP) in the P-mode was 10^2-10^3, and the minimum detectable amounts were 5×10^{-11} to 2.3×10^{-13} g/second.

The photoionization detector (PID) is in principle a flame ionization detector in which the ions are created by UV photons instead of a flame. Sensitivity is 10 to 50 times greater and the linear dynamic range is 10-100 fold greater than the FID. The PID is sensitive to inorganic compounds such as NH_3, PH_3, and AsH_3 to which the FID does not respond. The detector (83) has a sealed UV source focused directly into the ionization chamber. The UV energy photoionizes the various compounds eluting from the GC column but not the helium carrier gas. Organic compounds with ionization potentials greater than 10.2 ev, such as C_1-C_4 hydrocarbons including many common pesticide solvents (methanol, methylene chloride, carbon tetrachloride, acetonitrile), do not respond. The increased sensitivity is apparently due to the increased ionization efficiency of photons compared to a flame and operation of the PID in an oxygen-free environment, thereby eliminating free-radical quenching. The linear dynamic range has been reported as 10^7 - 10^8, and the minimum detection level was <2 pg for benzene. The PID response to carbon is proportional to the carbon number as is the FID. Because it responds to the sample concentration, maximum sensitivity is obtained at low flow rates (1-10 to 10-100 ml/minute). The maximum operating temperature is 250°C. Although this detector has not been evaluated for pesticide analysis, its high sensitivity suggests that it may be promising, especially in automated systems because of its great linear range.

Principles of the use and details of the arrangement of multiple detectors for efficient GC determination and confirmation of pesticides of different chemical types in a single sample are discussed in Section 320 of the FDA PAM, and a specific description of the combination of electron capture and thermionic detectors for simultaneous determination of OCl and OP pesticide residues is given in Section 321 of the same manual. When a combination of detectors is used, the chosen cleanup procedure must be suitable for the least selective of the detectors, enough residue must be injected to meet the minimum sensitivity of both detectors, and the nature and amount of injected solvent must be compatible with both. A GC system with one column, a three-way effluent splitter, and five different detectors (electrolytic conductivity (N-mode), FPD (FID, S-, and P-modes), and electron capture) operating simultaneously was described (84). A computer program for evaluating data from this system was later published. The program calculates retention times relative to two internal standards as references and peak areas corrected for baseline drift (85).

5I ELECTROMETER AND RECORDER

The electrometer is primarily a device for amplifying the electrical signal from the detector prior to its introduction to the recorder. Units may be single channel, designed to operate with one detector and recorder, or dual channel. Customary controls on the electrometer include input and output attenuators, output polarity, and controls for the recorder zero and bucking current. Servicing of electrometers is generally a function of a trained electronic technician or representative of the company manufacturing the chromatograph.

To check electrometers on the Tracor MT-220 chromatograph, set attenuators to the off position and zero the recorder. Set the output attenuator at x1 and record the baseline. A steady baseline with less than 1 percent noise should be obtained.

Recorders may be of single or dual channel design, the latter being capable of receiving two separate voltage signals supplied from the electrometer to two pens which trace separate chromatograms on opposite sides of the same chart paper. Electronic controls on a recorder usually consist of a pen zero and a signal gain adjustment. Most chromatographs require a recorder with a full scale sensitivity of 1 mv and a full scale response of one second or less.

Proper adjustment of the recorder gain control is extremely important. Some analysts, upon observing excessive baseline noise, erroneously conclude that this should be eliminated by lowering the gain. When the gain is set too low, however, the resulting chromatograms appear "terraced" with a stepping-stone effect in the baselines. In extreme cases, peaks have jagged and flat rather than pointed tops. When this is evident, correction can usually be achieved by advancing the gain control to a point just short of pen chatter.

5J SAMPLE INJECTION AND THE INJECTION PORT

a. On-Column and Off-Column Injection

Some gas chromatographs have injection ports designed to accommodate either on-column or off-column injection. The former entails insertion of the syringe needle directly into the glass wool inlet plug of the column. For off-column injection, some type of glass or metal insert is installed in the injection port, and injection is made into this insert where the sample is flash vaporized and swept into the column by carrier gas. In practical operation in a pesticide laboratory that is injecting a heavy volume of biological extracts, off-column injection through a glass insert is preferable. A significant amount of extraneous material that would otherwise be injected directly into the column is trapped by the insert. If your chromatograph does not provide the option of off-column injection, it is mandatory to frequently change the glass wool inlet plug. The frequency of change is determined by daily monitoring of the extent of *p,p*'-DDT conversion (see 4F of Section 4). The plug is changed when the combined areas of the breakdown peaks (DDE and/or DDD) exceeds 3 or 4 percent of the sum of the areas of the *p,p*'-DDT and the breakdown peaks.

An earlier discussion of some problems associated with injection of unclean samples and maintenance of the injection sleeve was presented in Subsection 4F. Glass injection sleeves are cleaned in chromic acid cleaning solution, rinsed with water and acetone, and dried. A final silanization treatment of the clean injection sleeve with Supelco's Sylon-CT has also provided a dramatic solution to p,p'-DDT breakdown problems. The label instructions were followed.

There are known instances where changing the insert, glass wool plug, and even the first 1/2 inch of packing have not diminished conversion of DDT resulting from massive injections of uncleaned samples without proper on-going maintenance. Final correction required disassembly of the entire injection port and wire brush cleaning of all metal parts to remove encrusted filth. Following this, each part was further cleaned in an ultrasonic cleaner in alcoholic KOH and finally acetone rinsed. Some analysts recommend the use of a small plug of quartz wool in the exit end of the injection insert to act as a further trap for extraneous contaminants.

b. Septums

A large number of different types of septums are available commercially ranging from inexpensive plain black or grey silicone rubber to more sophisticated and expensive sandwich types. Excellent results have been obtained with the blue silicone rubber materials marketed by Applied Science Laboratories as their "W" series.

Bleed from seven types of freshly installed septums has been reported (86) for their first 2-3 hours of use. Although this did not cause loss of aldrin and endrin studied as model pesticides, precautions should be taken to avoid bleed by rinsing the septums with acetone, preconditioning in a vacuum oven for two hours at 250°C, and storing until use in an oven at 130°C. Another option is to routinely change the septum at the end of each day to allow overnight purging of the system of any contaminant bleed. Still another approach is to install a septum shield, which in effect removes the septum from the injection system except at the very moment of injection. The paper describing this device as a remedy for septum bleed also studied the appearance of extraneous peaks due to the flow controller and the carrier gas as well as design of the injection system (87). Handling of septums with the fingers should be avoided.

Applied Science Laboratories HT blue septums are made from an especially low bleed silicone rubber and require no extensive preconditioning steps. The septum is rinsed with acetone, wiped with a Kimwipe tissue, air dried, and inserted using the tissue or clean forceps. Both the W and HT septums are indefinitely stable below 250°C and will typically withstand at least 50 injections with a 26-gauge needle in good condition.

c. Injection Techniques

(1) Handling the Syringe

When a sample is injected into the chromatograph, it is essential that it be entirely vaporized without loss. Injections are usually made using a 10 µl syringe for the electron capture, thermionic, and FPD detectors or a larger capacity if required for other less sensitive detectors (e.g., microcoulometric). Automatic injection devices are available for use with some chromatographs and detectors. (See Section 5Oj).

Samples are injected from a microliter syringe by inserting the needle through the septum as far as possible, depressing the plunger with the thumb or finger, then immediately withdrawing the needle (keeping the plunger depressed) as rapidly and smoothly as possible. Some analysts prefer a delay of 1-2 or up to 5-10 seconds before withdrawal of the needle. When initially filling the syringe, air is expelled by repeatedly drawing liquid in and rapidly expelling it with the needle tip still under the liquid surface. The volume of sample to be injected is exactly adjusted by drawing up a couple of µl more than necessary into the barrel. Hold the syringe vertically with the needle pointing up, put the needle through a tissue to absorb expelled liquid, and push the plunger until it reads the desired value. The excess air should now have been expelled.

There should be no delay between filling and injection of the sample. After injection, the syringe is rinsed clean by filling with and expelling 5 portions of ethyl acetate or acetone, and the syringe is pre-rinsed with the next sample to be injected in the same manner. Be sure to follow carefully all manufacturer's suggestions for proper use of each particular syringe.

When a sample is injected in this normal manner from a 10 µl syringe, the needle will retain ca. 0.2-0.3 µl of sample. It is usually safe to ignore this volume since standards are injected for comparison and the errors due to retained volumes will cancel out if equal volumes are used and concentration differences are negligible. Alternatively, the syringe may be filled by drawing the entire sample into the barrel, noting the final volume by reading each end of the column of liquid. After injection, the plunger is pulled back and the small volume of retained solvent now in the syringe barrel is read and applied as a correction. This will correct for nonreproducible injection technique but not, however, for the error encountered if the retained volume has a composition different from the original sample, as would happen if nonuniform distillation had occurred in the needle. Then the remaining liquid would be richer in high boiling sample components.

This can be overcome by using the solvent flush injection technique, the most reliable and reproducible method available. About 2 µl of solvent is first drawn into the syringe followed by a 1-2 µl air pocket and then the required volume of sample. The sample is brought completely into the barrel so its volume can be read. On injection, the flush solvent behind the sample ensures injection of the entire sample without loss due to hang-up. Whatever method the analyst chooses to employ, he must be as consistent as possible in his injections of standards and sample. It is critical that the solvent chosen for injection of the sample completely dissolves the residues of interest, and the same suitable solvent should be drawn first into the syringe for the flush technique. The suitability of the solvent should be verified by obtaining reproducible peaks from repeated injections of a sample dissolved in the solvent.

It is good practice to reserve one syringe only for electron capture work. If a series of concentration levels is to be injected, the more highly concentrated solutions should be injected last. If the complete freedom of a presumably clean syringe from pesticide traces is suspect, pure solvent should be injected and any peaks would indicate contamination and need for further cleaning. Dirty syringe plungers and needles should be wiped with lint-free wipers dipped in an appropriate solvent (e.g., ethyl acetate), and the barrel should be cleaned by drawing solvent through the needle and out the top with a vacuum.

(2) Preferred Volume Range

Injection of 1-3 µl samples is not recommended because of the large increase in error probability resulting from these small volumes. For example, a typical absolute injection error of 0.2 µl in a 1.0 µl injection would produce a $\frac{0.2}{1.0} \times 100 = 20\%$ relative error, while the same 0.2 µl error in a 5 µl injection volume reduces the relative error to a tolerable 4 percent level. The analyst is strongly urged to inject volumes between 5 and 8 µl from a 10 µl syringe for analyses with electron capture detectors. A syringe filled close to capacity is more difficult to manipulate. Proper dilution of standards and samples will provide on-scale peaks upon injection of optimum volumes. Standard and sample solutions are prepared so that peaks of approximately the same area are produced (Subsection 5O). A capable analyst should be able to reproduce a series of 3-8 µl injections to within 1-5 percent of average peak area or height when response is ca. 1/2 fsd with use of proper techniques.

The preceding paragraph describes the conventional wisdom concerning normal use of a common 10 µl GC syringe. Data have been presented, however, leading to recommended volumes between 2-4 µl. Below 2 µl, the error of injection increased above the ± 4-5 percent range. Reproducibility decreased for samples greater than 4 µl, supposedly due to the difficulty in quantitatively transferring the total volume from the syringe because the piston sealed poorly and allowed the liquid to be forced back or leak through the back of the syringe (87).

5K ERRATIC BASELINES

If all modules of the GC system are functioning properly, baseline noise should be below 1% fsd. When noise exceeds this level with the electron capture detector, all analytical work should be suspended until the cause is isolated and corrections made. A poorly regulated current supply or column liquid phase bleed can cause an erratic baseline. The slightest leak anywhere in the flow system may permit the entry of air and can be another cause of a noisy baseline. The most common points of leakage are probably septums which are not changed often enough or loose column connections. When a column is installed, fresh O-rings should be used. Satisfactory results have been obtained with heat-resistant silicone rubber column O-rings (temperature limit 300°C) used with brass ferrules. A reversed back ferrule has traditionally been used between two O-rings to cushion the glass from thermal shock, but one O-ring with no back ferrule is recommended by one commercial source of the silicone rings. Even though the Swagelok nuts are tightened securely on a cold column, an overnight period at normal operating temperature may result in sufficient loosening to cause a leak. When fresh O-rings are installed, it is good practice to open up the oven after the first overnight heating period and retighten the nuts.

Currently, three types of ferrules are most highly recommended for glass column pesticide work, and the choice appears to be mostly a matter of personal preference. Teflon, graphite, and Vespel polyimide-graphite combination ferrules are all available in one piece design for use without a brass ferrule or any O-rings (also reducing ferrules for use without reducing unions).

All are reuseable if carefully removed from old columns. Temperature limits are 250°C for Teflon, 450°C for graphite, and 350-450°C for Vespel. As with other Swage-type fittings, the nut is tightened until finger tight and then a further 1/4 to 1/2 turn with a wrench until leak tight. The need for further tightening should be checked after the initial heating period.

When temperature programming is employed to facilitate complex separations, dual column GC operation will compensate for the baseline of the analytical column. The dual columns contain the same liquid phase but need not be the same length. To set up the desired baseline, the recorder and detector are first zeroed. The columns are then heated to the upper temperature limit of the program where the bleed from the columns will be greatest. The resultant baseline is adjusted to the desired baseline by varying the flow rate of the balancing column. Another approach for "high bleed" analytical columns is to place a short, "low bleed" scrubber column (e.g., a low loaded silicone) at the analytical column exit.

5L RECOMMENDED GC COLUMNS FOR PESTICIDE ANALYSIS

a. Column Selection

A number of important factors must be considered in the choice of a column or combination of columns most suitable for a particular laboratory. Some of these factors are the following:

(1) The selected columns should be capable of separating the largest number of pesticides of interest with a minimum number of overlapping peaks. For example, 10% DC-200 or 3% OV-1 non-polar methyl silicone columns are of limited value to the analyst determining the more common chlorinated insecticides in environmental or animal samples. Partially or completely overlapping peaks are obtained for several pesticides generally detected in these sample substrates, e.g., p,p'-DDE and dieldrin, o,p'-DDT and p,p'-DDD, and the isomers of BHC (Figure 5-M,A).

(2) A high efficiency column is desirable if injected extracts contain extraneous materials and detection of low pesticide concentrations is required. This will provide sharper separation between the peaks of interest and extraneous peaks of biological origin.

(3) Retention time or elution speed may be a primary consideration if the analyst is concerned only with quantitation of certain, specific pesticides. For example, in a project where the sole interest is to routinely determine residues of a late-eluting pesticide such as methoxychlor, the column selection and operating parameters would be tailored to elute methoxychlor in a minimal time period consistent with its separation from any extraneous peaks.

(4) Pairs of working columns should be selected to be of dissimilar polarity and therefore provide different elution patterns ("fingerprints") (Subsection 5N).

(5) Shorter columns may be adequate for chromatography of certain, late-eluting pesticides, but for multiresidue analysis of unknown samples, a 6-foot column is recommended to obtain optimum efficiency and peak resolution.

b. Phases Used in the EPA Laboratory Network

After a careful comparative study of many GC columns with the above factors in mind, the four liquid phases listed in Table 5-3 were chosen as working and confirmatory columns for the routine analysis of organochlorine insecticides in human tissues. These columns will efficiently separate the principal compounds of interest (DDT, DDD, DDE, BHC isomers, heptachlor epoxide, and dieldrin) in a reasonable time, have low bleed, and give long service when properly prepared, used, and maintained, as described in Section 4 of this Manual. They have also proven to be excellent columns for general use in the determination of many pesticide classes in various substrates. The SE-30/OV-210 and OV-210 columns are especially recommended for separation of organophosphorus pesticides to be detected with the FPD.

Each of these phases has its own peak elution pattern for the compounds of a given mixture. An efficient column of the mixed phase OV-17 (phenyl methyl silicone) with QF-1 or OV-210 (trifluoro propyl methyl silicone) separates all usual tissue peaks completely except for a ca. 75 percent separation between p,p'-DDE and dieldrin. Higher load mixtures must be operated at very high temperature and carrier gas flow velocity to avoid slow elution and are not recommended. The SE-30 methyl silicone/OV-210 column gives no separation between lindane and β-BHC but good separation between dieldrin and p,p'-DDE on an efficient column.

TABLE 5-3

RECOMMENDED LIQUID PHASES FOR PESTICIDE ANALYSIS

Phase	1.5% OV-17/ */ 1.95% OV-210	4% SE-30/ */ 6% OV-210	5% OV-210	3% DEGS
Solid Support	Chromosorb W, H.P. or Gas-Chrom Q, 100/120 mesh	Chromosorb W, H.P. or Gas-Chrom Q, 80/100 mesh	Chromosorb W, H.P. or Gas-Chrom Q, 100/120 mesh	Gas-Chrom P 80/100 mesh
Approx. Operating T, ^{o}C	200	200	200	195
Approx. Flow Rate, ml/min.	50-70	70-90	45-60	70-90

*/ Individual liquid phases premixed prior to coating on silanized support

The single phase OV-210 gives a full separation of the common BHC isomers, but only fair separation between the compound pairs of heptachlor, epoxide/p,p'-DDE and p,p'-DDD/p,p'-DDT. The single polyester phase DEGS gives excellent separations of BHC isomers, complete peak separations of all compounds usually in tissues, and an unusual peak sequence (β-BHC after o,p'-DDT and p,p'-DDT before p,p'-DDD) which makes it useful for confirmation of peak identities. The DEGS column, however, bleeds and degrades easily and has a relatively short column life. It is therefore not recommended as a routine working column, but only as a special purpose identification tool.

Chromatograms of standard chlorinated pesticide mixtures on these columns and a single phase nonpolar DC-200 column are shown in Figure 5-M. Relative retention times of over 60 chlorinated and phosphate pesticides on OV-17/QF-1 */, SE-30/OV-210 and OV-210 columns between 170 and 204°C are listed in Subsection 4,A(6) of the EPA PAM and data on these columns also appeared in the literature (89, 90).

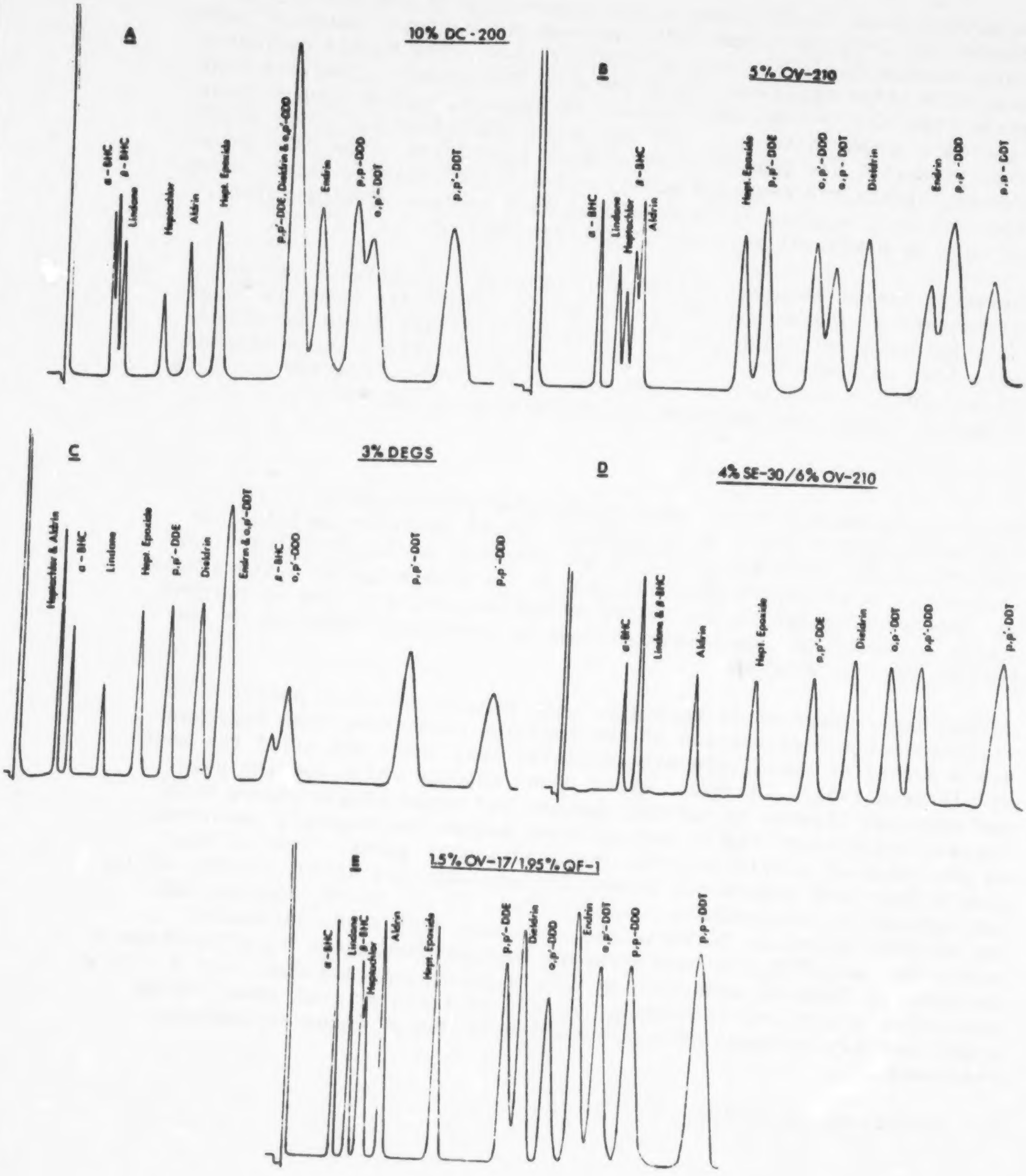
c. Other Pesticide Columns

Many other phases besides those recommended in Subsection b are available commercially. Some of these may be entirely satisfactory for residue analysis while others are outdated or unsatisfactory for the task. As suggested in Subsection a, a wide range of factors must be considered in making the column selection, and a column or columns wholly suitable for one laboratory may be completely unsuited to the typical work of another.

In the early years of GC analysis, only single, nonpolar phases were utilized for the separation of the nonpolar pesticides then important, and a survey of the literature indicates that these are still the most widely used today. However, mixed phase columns which combine polar and nonpolar liquids in varying degrees and newer single phases with varying polarities (the OV series) have become increasingly important as the range of pesticide types has drastically grown. Some of the more widely used additional phases include nonpolar SE-30, DC-200, DC-11, and Apiezon L; intermediate polarity QF-1, OV-17, XE-60, Reoplex 400, and DC-550; and polar Carbowax 20M, Versamid 900, NPGS, butanediol succinate, and NPGA. Common supports besides Chromosorb W or Gas-Chrom P include Gas Chrom Q, Anakrom Q or ABS, Supelcoport, and Diatoport S. In a particular analytical situation, any one of these or some other column might possibly be equal or even superior to one of those recommended previously.

*/ equivalent to OV-210

Figure 5-M. Peak elution patterns of 13 pesticides on five columns.



The U.S.F.D.A. continues to recommend 10% DC-200 and 15% QF-1/10% DC-200 columns at 200°C with a carrier flow of 120 ml/minute in their multi-residue determinative methods for foods (FDA Pesticide Analytical Manual, Chapter 3), even though other lower-bleed liquid phases used with lower loading and slower flow rates provide greater response and resolution. Relative retention data for numerous compounds under these conditions are listed in the FDA Manual. FDA's primary mission is that of testing for compliance with established tolerance, generally expressed in terms of parts per million (ppm). In view of these relatively high concentration levels, the use of highly responsive and efficient columns is not as critical as in the case of laboratories testing in the ppb and ppt range. Chapter 3 of the FDA PAM also contains extensive data on columns containing 2% DEGS (200°C, 60 ml/minute), 15% QF-1/5% DC 710 (2:1) (200°C, 100-200 ml/minute), 15% OV-210 (190°C, 80 ml/minute), and 10% OV-101 (200°C, 120 ml/minute). Other recommended liquid phases include SP-2100 (silicone), SP-2401 (50 percent trifluoropropyl substituted silicone, similar to QF-1 and OV-210), HI-EFF-1BP (similar to DEGS), and OV-11 (35 percent phenyl substituted silicone, similar to 50 percent phenyl substituted silicone DC-710).

The Canadian Department of National Health and Welfare (91) specifies 6 foot x 1/4 inch columns of the following single phases coated at a 3 percent level on Chromosorb W, AW, or HP for their multiresidue monitoring procedures: OV-1 (nonpolar), OV-17 (slightly polar), OV-225 (medium polar), ethylene glycol adipate (polar), and DEGS (very polar). The relative polarities were calculated from McReynolds constants (92). The 4% SE-30/6% QF-1 mixed phase is also recommended. A particular phase is chosen according to the polarity of the pesticide(s) of interest. Relative retention times are listed (93) for over 100 pesticides on OV-1, OV-210 (intermediate polarity), DEGS, and mixed phase columns.

The mixed phases OV-1/OV-17, OV-210/OV-17, OV-225/OV-17, OV-1/OV-25, OV-210/OV-25, and OV-225/OV-25 have been recommended for separation of organochlorine insecticides, and tables of relative retentions were given for 14 compounds (94). A column packed with three silicone stationary phases, namely 2.5% OV-11 + 1% QF-1 + 0.5% XE-60 (phases premixed and pan coated on Chromosorb W HP), was shown to resolve a 14-component OCl insecticide mixture in less than 19 minutes; retention data were compared to other common pesticide columns (95).

Other extensive compilations of pesticide relative retention data appear in References (96-98).

In addition to the packed GC columns already discussed in this subsection 5L, the use of capillary columns is growing rapidly in analytical situations where high resolving power is required. The more common glass capillary column is the wall coated open tubular type (WCOT) where the liquid phase is distributed as a thin film on the inside wall surface without employing any support. Columns are generally 25, 50, or 100 meters in length x 0.25-0.75 mm id. The smallest diameter gives the best efficiency but lower sample capacities (typically 1-50 ng per component). Because of the low sample capacity, injection is accomplished by an injector-splitter where typically 1 µl is injected, 0.01 µl enters the capillary column, and 0.99 µl is vented. Carrier gas flow through such columns is ca. 1 ml/minute, and a make-up gas system is required to sweep any void volumes and optimize detector flows. The major advantage of capillary columns is the high total number of theoretical plates obtainable (plates per meter length are comparable with packed columns) with these long, high permeability (low back pressure), open tubes leading to tremendous separation efficiency for complex environmental samples. The thin liquid film thickness provides fast analysis times, often at relatively low temperatures, and sharp peaks. Also available are support-coated open tubular (SCOT) columns, where a layer of support (e.g., Celite) is adsorbed on the tubing wall and a liquid phase is adsorbed on the support. SCOT columns have increased capacity, wider tubing (ca. 0.02 inches), and faster flow rates (4-10 ml/minute), and dead-volume connections are less critical than in a WCOT column. Sample splitting is often used but not required (sample normally <0.5 µl). Capillary columns are expensive and require good technique and instrumentation, but they are invaluable for separations requiring a large number of theoretical plates. See Reference (99) for a review of capillary GC. Capillary columns have been mostly used in pesticide analysis for the GC-MS identification of PCBs and chlorinated pesticides (Section 7AC). In addition, retention data were reported for 60 organophosphorus pesticides and for 27 chloro-, bromo-, and nitrophenols on SE-30 capillary columns (54). The combination of an OV-101 capillary column with a pulsed mode EC detector was evaluated for quantitation of lindane, and a minimum detectable amount of 1 pg and linear response to 2.4 ng were found (100).

5M SENSITIVITY OF THE GC SYSTEM

For analysis of pesticides in environmental media, concentrations of residues are commonly in the ppb or ppt range. High sensitivity of GC detection is therefore an obvious requirement. This is not usually so important a factor in a laboratory primarily oriented to enforcement of statutory tolerance levels in agricultural commodities or foodstuffs, since tolerance levels are usually set in the ppm range. For analyses of environmental media, the various electrical gas flow, and temperature parameters must be optimized to produce a peak at least 50 percent full scale deflection (fsd) (with minimal baseline noise) from injection of 100 pg aldrin on one of the four recommended columns (Subsection 5L) connected to an electron capture detector. Other sensitivities (1/2 fsd) should be approximately as follows: 2.5 ng ethyl parathion for the FPD (P Model), 25 ng diazinon and 35 ng parathion for the Coulson conductivity detector (N Mode).

The foregoing subsections survey sensitivities reported for the other pesticide GC detectors. When adjusting parameters to achieve optimum response, it should be recalled that signal to noise ratio is a more meaningful definition of sensitivity than is peak height alone. It has been found in many instances that a significant improvement in sensitivity (and concurrently in column efficiency) can be achieved by simply lowering the carrier gas flow rate.

A sample extract volume of 10 ml from a 5 gram sample contains the tissue equivalent of 0.5 mg/µl. A 5 µl injection of this extract (2.5 mg of sample) into an electron capture detector should easily produce quantifiable peaks at pesticide concentrations of at least 0.1 ppm provided sensitivity is adequate and attenuation is appropriately adjusted. The high sensitivity capability of the chromatograph should be utilized by optimization of parameters to permit operation at low output attenuation. It is poor practice to operate the electrometer at high attenuations (10 x 32 or 10 x 64 on the Tracor MT-220) while adjusting standard and sample concentrations to fit this attenuation range. With a new detector foil, high attenuation may be necessary, but in general this practice, although giving chromatograms with a stable baseline, requires injections of relatively high sample concentrations to produce quantifiable peaks. This leads to more rapid contamination of the column and detector than would result from injection of less sample material, a consideration that is particularly important when injecting the 15 percent ether-petroleum ether Florisil column eluate from a fat sample (Subsection 7Aa). If the instrument is functioning properly, it should be possible to have a noise level not exceeding 2 percent full scale at a low signal attenuation (10 x 8 or 10 x 16).

It is important to distinguish between the terms *sensitivity* and *limit of detection*. Sensitivity is the amount of compound necessary to obtain a certain response from an instrument under a given set of instrument parameters. At maximum useable sensitivity, the response (e.g., peak height) for the compound should be at least twice the response value of the noise (101). Sensitivity can be expressed as the absolute amount of compound providing the defined response or in relative terms, such as peak height or area for a given weight of compound. Limit of detection is the concentration of pesticide above which a given sample of material can be said, with a high degree of confidence, to contain the chemical being analyzed by a definite, complete analytical procedure (102). The value depends upon the pesticide and the substrate and is expressed in relative units such as ppm or ppb.

5N QUALITATIVE ANALYSIS

In analyzing a sample extract, the first step, after appropriate cleanup and concentration, is to run a preliminary chromatogram. Assuming the chromatography system is operating under the type of control already discussed (e.g., the actual column temperature is known from the RRT_{A} *p*,*p*'-DDT), relative retention data can be related to tables (EPA PAM, Subsection 4,A,(6)) for the particular column and temperature to make tentative peak identifications. If data indicates one or more probable pesticide peaks, proper standard mixtures are selected and quantitation is carried out as described in Subsection 5O. Confirmation of peak identity is obtained by chromatography on alternate columns and/or an alternate selective detector, or by another chromatographic (e.g., TLC) or non-chromatographic procedure (Section 8.).

Both *absolute* and *relative retentions* have been used for qualitative analysis of pesticides. Absolute retention is the actual time between the injection of the sample and the elution of the peak. On a chromatogram, the measurement is usually made in millimeters between the injection point or the front of the solvent peak to the maximum of the peak of interest (distance x, Figure 4-C, Section 4.). Conversion of retention to minutes is easily made if the chart speed is known. With detectors such as the microcoulometer which do not respond to the solvent, the injection point must be manually or electrically marked to serve as a reference point, and this must be done with accuracy.

Relative retention ratio is the ratio of the absolute retention of the compound of interest to that of a reference compound, most commonly aldrin or ethyl parathion. For peaks which elute before the reference, the relative retention time will be less than 1.0; for those which elute after the reference, the relative retention time will be greater than 1.0. When reporting relative retention data, the absolute retention time and relevant instrumental parameters should be given.

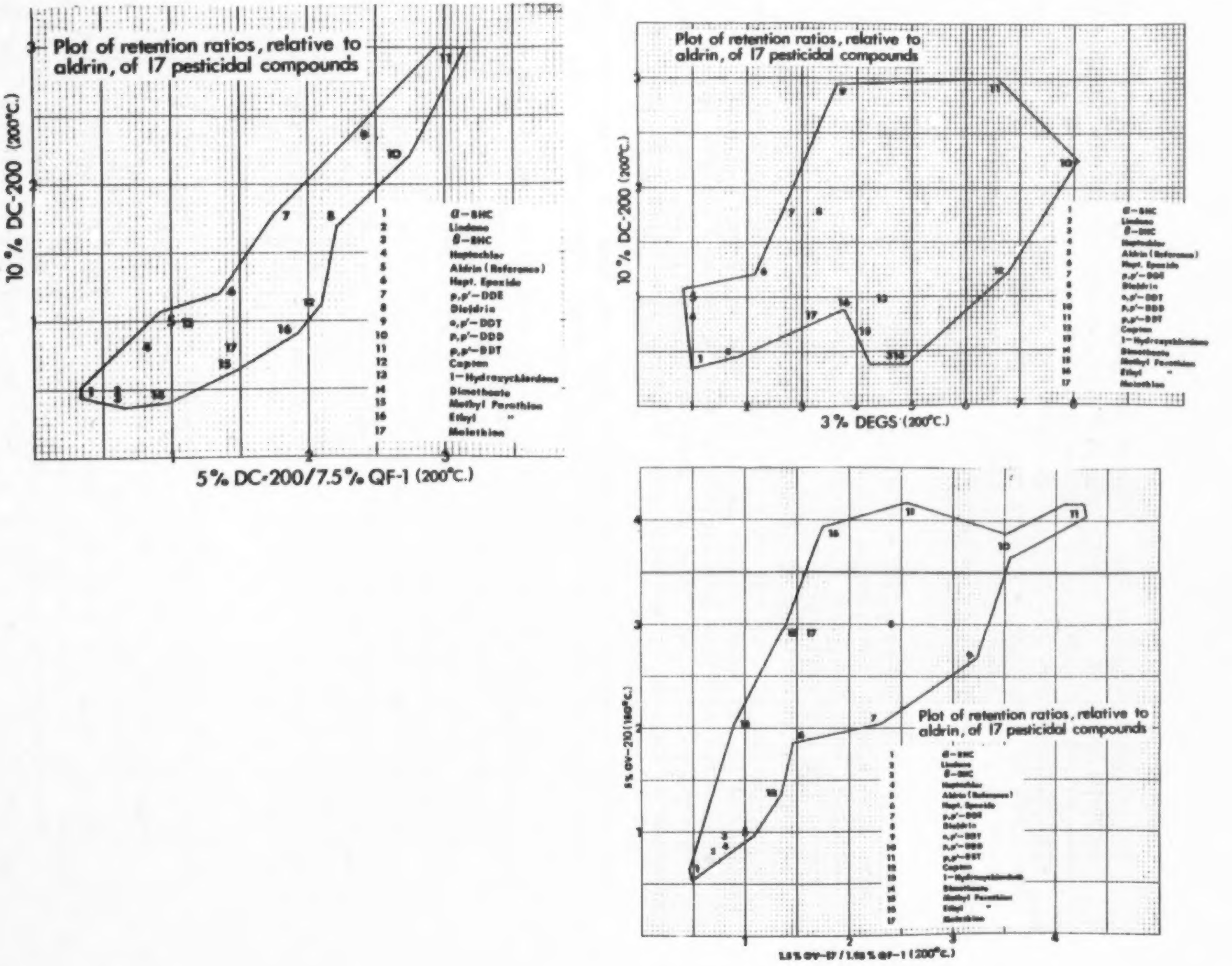
The relative retention ratio is far more reproducible than the absolute retention value since only the column temperature will influence the former. Absolute retentions can vary slightly from day to day or even from hour to hour. The reference pesticide may be chromatographed just before or just after the sample, or it can be added to the sample so that its peak will appear on the same chromatogram. This latter approach is preferred if the sample is known to contain no compounds producing a peak with the same retention time as the added reference compound. In addition to relative retention, peak geometry (shape) is often an additional useful aid in comparison of sample and standard chromatograms.

Although confirmation will be treated in detail in Section 8, some comments pertaining to compound identification will be made here. The most common single factor in failure to properly identify a pesticide is the use of only one GC column. It is impossible to be sure a given column has separated all pesticides present in an unknown mixture, and if this does occur it is the result of an extreme case of good luck. Reliance on a single column is totally unacceptable and will usually lead to worthless analytical data, both qualitative and quantitative. If two columns are to be used, they should be judiciously chosen to be entirely different in their elution patterns. Complementary pairs of columns include OV-17/QF-1 with OV-210, and SE-30 with DEGS.

Elgar (103) ingeniously illustrated this point by demonstrating that when two similar columns are used and the relative retention ratios for a number of pesticidal compounds are plotted on respective axes, the points fall on a relatively straight line with little scatter in evidence. Conversely, when two dissimilar columns are used, the plotted points show a wide scatter, enhancing the probability of reliable identification. Figure 5-N shows the plots of three column pairs for 17 pesticidal compounds detected by electron capture. A is the plot of 10% DC-200 vs. 5% DC-200/7.5% QF-1, B is 10% DC-200 against 3% DEGS, and C is 5% OV-210 against 1.5% OV-17/1.95% QF-1. It will be observed that the RRT points plotted in A cluster to an extent that a fairly straight line is represented by the plots. Plots B and C, on the other hand, show a very wide scatter, indicating that either of these two pairs is an excellent choice for complementary columns.

High column efficiency is a distinct advantage for compound identification in that pesticides will be well resolved from each other and from non-pesticide artifacts coextracted from the sample substrate. In addition, operating parameters must be adjusted to produce the most decipherable chromatograms. For the columns recommended in Subsection 5L, the oven temperature should be set so that p,p'-DDT elutes in 16 to 18 minutes with a carrier gas flow of 50-80 ml/minute. The recorder chart speed

Figure 5-N. Plots of retention ratios, relative to aldrin, of 17 pesticidal compounds on three column pairs.



is set to permit adequate peak spacing and a total retention distance such that an absolute measurement error of 1.0 mm will correspond to an insignificant relative error. These precautions will help assure good peak resolution and precise retention measurements.

A computer-plotting program has been described that can serve as an aid in qualitative analysis of pesticide residues (104). Chromatograms are reproduced with corrected baseline drift and solvent peak elimination, and two or more chromatograms can be presented in a three-dimensional view to facilitate rapid visual comparison to determine whether there are differences in the characteristics of individual peaks (sample or standards) between chromatograms.

50 QUANTITATIVE ANALYSIS

a. Introduction

Quantitation of pesticide residues known to be present in the sample from relative retention data and various confirmatory procedures is carried out by comparison between the size (height or area) of the peak for each pesticide in the sample and the size of a peak from a similar, known amount of each compound injected under the same GC conditions just before and/or after the unknown sample. Only one standard concentration is required for each unknown if injections are made at concentration levels providing linear detector response. This procedure is known as the external standardization method.

The exploratory chromatogram of the sample extract used to obtain relative retention data will provide a tentative indication to the analyst of the proper standard mixture to be used. This mixture should contain the pesticides of interest at proper concentration levels to fall within the linearity range of the detector and also to produce peaks comparable in size to those obtained from the sample chromatogram. Injection of the standard mixture may show that additional dilution of the sample extract is required to produce peaks of the higher concentration pesticides comparable to those from the standard mixture. If several standard mixtures are available at different concentration levels, selection of one closely approximating the unknown will facilitate the analysis (Subsection 50f).

b. Comparison of External and Internal Standardization

Internal standardization is a widely used, general analytical and gas chromatographic technique which, however, is not recommended for multiresidue pesticide determinations. Since multiresidue methods can detect and measure a large number of different compounds, choice of a suitable standard with appropriate structural and chromatographic properties in terms of all compounds to be quantitated would be an impossible, or at least a very difficult task. Response calibration for all compounds of interest vs. the internal standard would be a lengthy process and would require frequent checking. To determine the amount of internal standard to add, a preliminary analysis of samples with unknown histories and compositions would be necessary. Many samples require gas chromatography at several dilutions to quantitate all residues, so different quantities of internal standard would be required. Detector response to sample coextractives further complicates the choice of an internal standard. These and other disadvantages dictate against the use of internal standardization except in special cases, such as the analysis of pesticide residues of one or a small, definite group of pesticides.

External standardization has the advantage that calculations are based on a comparison of the same compound in the standard solution and in the unknown, and no response or correction factor is required. Accuracy and precision depend upon the ability to inject exact amounts of samples and standards reproducibly, having all instrumental parameters under tight control so that data are comparable from run to run and determinations being conducted within the linear concentration range.

A recent study (87) concluded that generally unrecognized systematic errors were inherent in the accepted procedures of both external (direct) and internal standardization GC. For example, it was found necessary to consider both the volume injected and the concentration of the standards in the direct method; plotting peak area vs. quantity (gram, mole) is not sufficient unless the concentration is stated and the volume is kept constant. The internal standard method was found to be not necessarily independent of the volume injected, concentration of standard, or the effects of temperature and gas flow on instrumental sensitivity. The relevance of these conclusions to pesticide analysis has not been studied, and the procedures recommended in this section are based on the best current practice of experienced residue analysts.

c. Calculation Procedure

A universal equation applicable for any GC analysis where an unknown peak is calculated against a peak resulting from injection of a standard of known concentration is given below. This equation is equally applicable to external standardization procedures based on comparison of standard and unknown liquid chromatography peaks or thin layer chromatography spot sizes. The equation is:

$$R = \frac{abe}{cd}$$

where a = nanograms of pesticide represented by the standard peak

b = height (or area) of sample peak

c = height (or area) of standard peak

d = grams (or ml) of original sample

R = residue concentration in parts per million or billion

e = Dilution Factor derived as follows:

$$e = \frac{\text{ml of extracting solvent} \times \text{volume of final extract*}}{\text{aliquot taken of original extract (ml)} \times \mu\text{l injected}}$$

* This value is in µl for ppb and in ml for ppm

In calculating the Dilution Factor (e) for a situation where the final extract concentrate contains the entire original sample (where no initial aliquot is taken), the values for the "ml of extracting solvent" and the "aliquot taken of original extract" would cancel out, so that the e value would become

$$e = \frac{\text{volume of final extract } (\mu\text{l or ml})}{\mu\text{l injected}}$$

The basic equation can be simplified when conducting routine, repetitive analyses. For example, considering a method such as the blood procedure outlined in Subsection 5,A,(3),(a) of the EPA PAM,

(1) d is always a constant size (2 ml)

(2) the volume of extracting solvent is always the same (6 ml)

(3) an aliquot of uniform volume is always taken (5 ml)

(4) a constant injection volume is always used (5 µl)

The equation can be simplified to match these parameters and would read

$$R = \frac{abf}{c} \times 0.12$$

where f = volume of final extract in µl or ml. The numerical factor 0.12 represents e for the specific illustration above.

d. Reporting of Results

The method for reporting analytical results will often differ from laboratory to laboratory, but in general, the following should be stated:

(1) The compounds or classes of compounds being sought.

(2) Other related or important compounds of interest that were detected or found absent.

(3) The limit of detection for each pesticide, as well as its degradation products and metabolites.

(4) Recovery values and whether results were corrected for recovery.

(5) The basis for selection of the analytical procedure and any modifications of an accepted procedure.

(6) Confirmatory methods.

Results should be reported in appropriate ppm, ppb, or ppt units, and the basis for reporting should be clear, i.e., dry weight, wet weight, or fat- or extractable-lipid basis. Any drying methods should be described. If replicates are run, the individual results, the mean, and a statistical treatment of precision (Section 2K) should be presented.

Pesticide residue analytical data are generally reported as ppm (parts per million), ppb (parts per billion), and ppt (parts per trillion). Converting these terms to weight expressions, we have

ppm = micrograms per gram

ppb = nanograms per gram

ppt = picograms per gram

Residues in water are quite commonly expressed as micrograms per liter, which is equivalent to ppb. On rare occasions a laboratory may choose to express a water residue result in grams per liter, but the value becomes quite cumbersome, i.e., 5×10^{-7} grams per liter as opposed to the more convenient 0.5 micrograms per liter.

The following is a summary of units frequently used in pesticide analyses:

µg = 10^{-6} grams ml = 10^{-3} liters

ng = 10^{-9} grams µl = 10^{-6} liters

pg = 10^{-12} grams

ppm = parts per million = µg/g, µg/ml, ng/mg, or pg/µg

ppb = parts per billion = µg/l, ng/g, ng/ml, or pg/mg

ppt = pg/g, pg/ml (ppt is used frequently in other books to mean "parts per thousand").

e. Detector Linearity

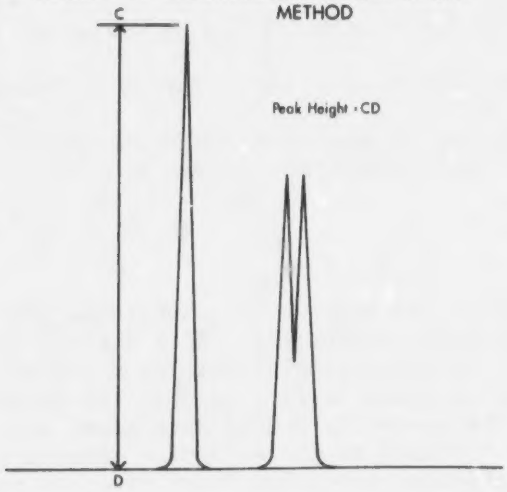
Linearity may be defined as the range of concentration over which a detector maintains a constant sensitivity. If a detector has a linearity of 10^3 and the detector sensitivity for a certain pesticide is 1 pg, the upper limit of analysis is 1 ng. If the detector sensitivity is 0.1 pg, the pesticide can be determined only up to 100 pg. Sensitivity is affected by the molecular structure and retention time for a particular pesticide under given GC conditions.

Quantitation must be performed within the linear response range of the GC detector. Each detector has its own characteristic linear range under the prevalent conditions of operation. For a given detector, the linear range varies somewhat between pesticides. For example, the isomers of BHC exhibit a more restricted EC linear range than p,p'-DDT. The nickel EC detector operated in the DC mode exhibits a far more restricted linear range than the tritium detector. Lindane concentrations above 600 pg may result in nonlinearity with the tritium detector, whereas the linear cut-off for this compound may occur at approximately 250 pg for the nickel detector. Figure 5-C compares typical linearity curves for p,p'-DDD with these two detectors.

Before any attempt is made to try quantitation with a new or newly renovated detector, linearity curves should be constructed for the pesticides of interest under the prevalent operating conditions. Frequent checks should be made to insure continued operation within acceptable concentration ranges. Knowledge of the linear cut off point will preclude such error as injecting 1 ng of aldrin and expecting it to fall within the linear range of the Tracor EC detector.

Calibration curves are constructed by injection of serial amounts of a pesticide and calculation of the peak height of each peak. Peak height is the perpendicular distance from the peak maximum to the baseline (Figure 5-0, distance CD). The linear range is observed by plotting height vs. amount of pesticide injected (Figure 5-C).

FIGURE 5-O QUANTITATION BY PEAK HEIGHT METHOD



f. Sensitivity Control

When analyses are performed in the ppb or even ppt (parts per trillion) range, electrometer attenuation is required to attain maximum sensitivity consistent with an acceptable baseline noise level. Electrometer operation at high sensitivity levels is good practice even when substrates contain high concentrations of pesticides. By serially diluting the final extract and operating at high sensitivity, the possibility of exceeding the linear range of the detector is greatly reduced, and therefore, the quantitative error possibility is reduced. Occasional instances have been observed in the EPA Quality Control Program where operators have set attenuation controls at low sensitivity and injected media containing massive concentrations of pesticides. This was readily discernable by malformed chromatographic peaks. Had attenuation been set for high sensitivity, chromatographic peaks would have gone off-scale, requiring serial dilutions of final extract to a pesticide concentration within linear boundaries.

Electron capture electrometer attenuation should be adjusted to obtain a *minimum* sensitivity level equivalent to a 50% fsd peak from the injection of 100 pg aldrin.

There is no objection to using different instrumental attenuation settings for standards and samples *provided* that concentrations are within the linear detection range and checks are made to insure that the attenuator is truly linear. A sample should produce the same peak when diluted by 10 as if the original sample were run at an attenuation increased by 10. An output attenuation setting of x16 on the Tracor MT-220 chromatograph electrometer is convenient to assure operation within the range of the detector.

g. Injection Volumes and Standards

As described in Subsection 5J, injection of small volumes such as 1-3 µl can lead to large relative errors and should be avoided. A common reason for low injection volumes is to provide on-scale peaks of sample for reference against peaks from a particular single standard or standard mixture. To circumvent this problem, standards should be made up at several concentrations, each succeeding level being twice the previous concentration. For example, a typical set of three standard mixtures in pg/µl for electron capture GC might be:

	Mixture A_1	Mixture A_2	Mixture A_3
Lindane	5	10	20
Aldrin	5	10	20
Dieldrin	10	20	40
o,p'-DDT	10	20	40
p,p'-DDT	20	40	80

Working standards must, of course, include all compounds of interest to a particular laboratory. It may be necessary to run several sample extract chromatograms of various concentrations and/or injection volumes to achieve reasonable concurrence of peak size with those of the standard mixture(s) if pesticides are present in the sample at widely different levels.

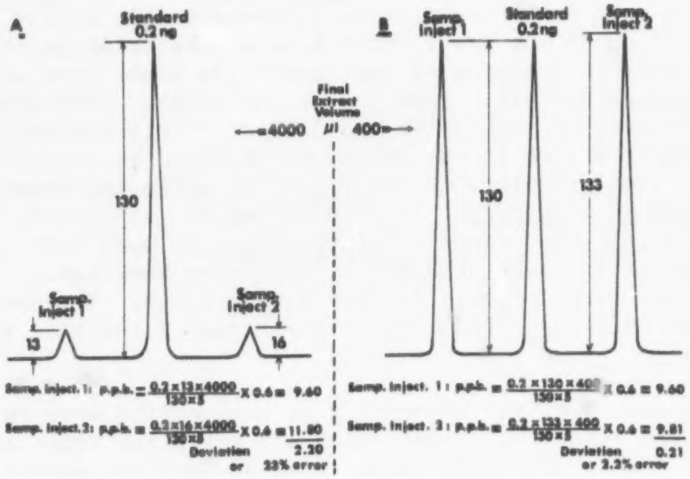
It should be unnecessary to reiterate that accuracy of analysis is limited by the accuracy of the standard quantitating solutions. Consistently high recovery values on an interlaboratory check sample strongly indicate that weak standard solutions are being used by the laboratory in question, while consistently low values indicate the probability of overconcentrated standards.

h. Optimum Peak Heights

The ideal range of peak height response is 20-60% fsd, with a minimum acceptable height of 10% fsd. Peak heights of the sample and standard should vary by no more than 10 percent for highest accuracy and at most by 25 percent. If all GC modules are operating properly and parameters are optimally set, the 10 or 20 percent fsd minimum peak requirement will cause no problem in terms of attainable sensitivity when standard procedures and concentration steps as given in the EPA PAM are followed.

The requirement of referencing samples against standards differing by no more than 25 percent in peak height causes no inconvenience when the concept of different standard concentrations (Subsection 50f) is followed. This point is important, even though one is working within the linear range of the detector, because of minor variations in GC response, primarily arising from instrumental sources and/or from small injection errors. Figure 5-P illustrates the potential error that is possible from a peak height variation of as little as 3 mm, when attempting to quantitate a 13 mm sample peak against a 130 mm standard peak. This is shown in (A) on the left. The total deviation results in a relative error of 23 percent. On the other hand, when the extract is further concentrated down to an assumed final volume of 400 µl, the height of the sample peak is increased by a factor of 10 to 130 mm as shown in (B) on the right. The same 3 mm response variation at this level will result in a final relative error of only 2.2 percent, a very acceptable value.

Figure 5-P. Illustration of potential hazard of quantitating by comparison of small sample peak against large standard peak. A 3 mm peak height shift is assumed. Initial sample size 1.67 grams; injection volume 5 µl.



i. Standard Curves

GC calibration curves prepared by injection of standards are of little direct use in residue quantitation. Such curves are not valid for extended periods of time, as is the case for other analytical methods such as spectrophotometry, and so their preparation is an unnecessary, time consuming chore. A GC standard curve constructed for an EC detector on a given day at 9 A.M. may well be worthless by the afternoon of the same day, or even the same morning, if high lipid extracts causing a rapid depression in peak response are repeatedly injected. If such peaks are referenced against a standard curve prepared when response was normal, erroneously low results will be obtained.

The proper procedure for quantitation is to intersperse standard mixture injections throughout the workday with sufficient frequency to signal the onset of response fluctuations, and quantitative referencing is made against the interspersed standards. For maximum accuracy, injection of an unknown sample would be bracketed between standard injections made immediately before and after the sample.

j. Methods of Peak Measurements

Both peak heights and peak areas are extensively used for calculations of residue amounts. The preferred method of calculation depends on the shape of the chromatographic peak. Peak height is recommended for measurement of very small peaks or tall, symmetrical, fairly narrow peaks (<10 mm at the base) which have no obscuring overlaps. These are characteristic features of most pesticide peaks from an efficient GC column, especially those that elute early. Accurate calculation of the area of such peaks would be difficult because the slightest measurement errors in the narrow width would be magnified in the subsequent area calculation. Peak area as estimated from peak height x width at half height is recommended for separated, symmetrical, and fairly wide peaks. Triangulation is used for separated, unsymmetrical peaks or peaks on a sloping baseline. Triangulation should never be used on very narrow peaks. Extreme care must be taken in the construction of inflectional tangents in all measurements.

When peak heights are used, the assumption is necessarily made that operating parameters are closely controlled and retention times are very reproducible. Two consecutive injections of the same amount of compound should ideally result in two peaks with exactly the same retention time, width, and height. If chromatographic parameters (particularly column temperature) are not under strict control, the second peak may instead elute later or earlier than the first, resulting in a wider or narrower peak. However, the peak areas should be the same in both cases. For this reason, peak area or peak height x retention time is considered by many operators to be more reliable than peak height alone since slightly shifting peak positions will not be so important.

Figures 5-O, 5-Q, and 5-R illustrate the peak height, peak height x width at half height, and triangulation measurement methods, respectively. The two right-hand peaks in Figure 5-O are measurable by the peak height method because their overlap does not abscure the height of either peak. Peaks on a sloping baseline but too narrow to be triangulated can be measured by the peak height method. The distance KI would be used as seen in Figure 5-R. Peaks can be widened by using a faster recorder chart speed. Use of the planimeter is an alternate method for measuring unsymmetrical peaks, peaks on a sloping baseline, or total area of a series of incompletely resolved peaks. Precision will be improved by tracing the peak at least twice and taking an average value.

FIGURE 5-Q QUANTITATION BY PEAK AREA METHOD

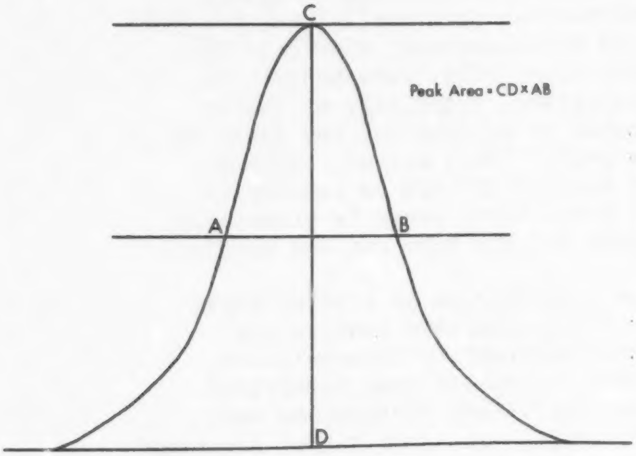
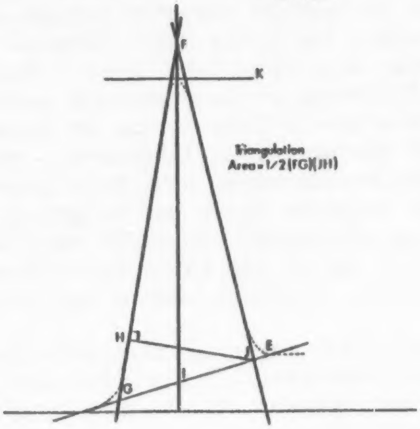
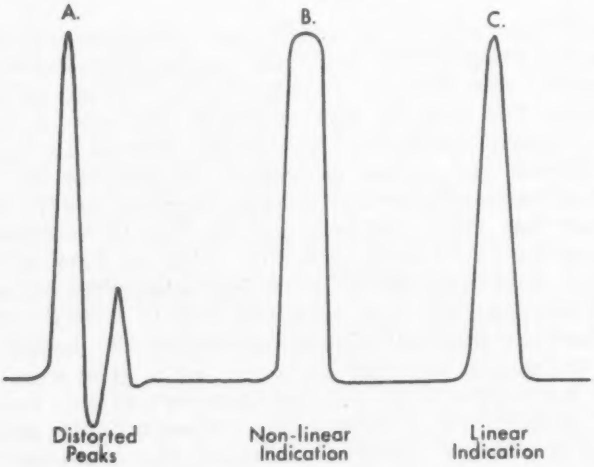

C
Peak Area = CD × AB
A
B
D

FIGURE 5-R QUANTITATION BY TRIANGULATION METHOD


F
K
Triangulation
Area = 1/2(FG)(JH)
H
J
E
I
G

Quantitation of peaks indicating heavy electrical overshoot (Figure 5-S,A) or nonlinear response (5-S,B) will lead to unreliable quantitation. Peak overshoot is influenced by foil contamination and by improper EC detector polarizing voltage (Subsection 5Cb).

FIGURE 5-S EXAMPLES OF GAS CHROMATOGRAPHIC PEAKS



Automatic (disc) integration is a convenient, accurate procedure that can be used in place of manual procedures whenever baselines are steady, but it is less reliable and simple when sloping baselines or peaks with shoulders occur. This method has been mostly used for calculation of late eluting peaks and multicomponent chemicals that elute over a long period of time (Strobane, PCBs, toxaphene). In the absence of an integrator, chromatograms, especially of these complex mixtures, have been quantitated by cutting out the peaks on the recorder chart and weighing the paper. This method, although time consuming, can yield excellent results if care in cutting is taken and if the paper is uniform. Since Xerox paper is especially uniform, recorder charts can be copied and the copy cut and weighed.

Gaul (105) compared five methods for quantitation of aldrin, heptachlor epoxide, and dieldrin with a tritium electron capture gas chromatograph. The methods were disc integration, triangulation, peak height times width at half height, retention time multiplied by peak height, and peak height. No significant differences were found among the five methods in this study. The same author described methods for properly placing baselines for typical overlapping and unsymmetrical gas chromatographic peaks, and suggested procedures for quantitating multipeak chromatograms of pesticides that are mixtures of isomers, e.g., DDT, BHC, chlordane, and toxaphene. Poorly resolved peaks and sloping baselines present the greatest challenge in terms of accurate quantitation, and an experienced analyst must exercise judgment to quantitate the peaks properly. If necessary, improved resolution of peaks and flatter baselines may be sought through the use of other cleanup procedures, GC columns, or changes in operating conditions.

k. Automation

Digital computer systems are available today that perform peak and baseline detection, area integration, baseline correction, area allocation of fused peaks, and postrun calculations. These relatively expensive systems provide the fastest and probably the most accurate means of quantitation. Their increased use in the future is expected as technology is simplified and prices decrease, especially in laboratories with many chromatographs and a heavy sample load. However, small laboratories may find complete automation impractical and inadvisable for complex biological samples. The various approaches for automation of sample introduction in gas chromatography have been reviewed and a typical autosampler described in detail (106). Besides the advantages of automation and unattended operation for large numbers of samples, automated injection systems will normally give more precise injection volumes than most operators can achieve manually. Losses of up to 50 percent of aldrin and dieldrin in a commercially automated dry capsule injector were reduced by silylation of the capsules (107).

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Section 6

ADDITIONAL PROCEDURES IN PESTICIDE ANALYSIS

This section treats a number of miscellaneous topics important in residue analysis. These include general considerations for collection and extraction of samples, methods for concentration of extracts, and determinations by thin layer and high performance liquid chromatography. Specific procedures for extraction and cleanup of pesticide and metabolite residues are discussed in Section 7.

SAMPLE COLLECTION, PREPARATION, AND STORAGE

6A GENERAL CONSIDERATIONS

Special consideration must be given to the procurement, storage, and transportation of samples to be analyzed for pesticide residues. Procedures should insure, as well as possible, that the pesticides originally present have not undergone degradation or concentration and that contaminating impurities which might interfere with the analysis have not been added. Plastics must be rigidly avoided as containers for samples to be examined by electron capture GC because minute traces of materials such as polyethylene may produce spurious responses. Similarly, metal containers may contain trace impurities such as oil films, lacquers, or rosin from soldered joints that will cause interference in GC analysis. In general, glass jars or bottles with aluminum foil or Teflon-lined lids are the most suitable sample containers, although it is sometimes possible for pesticides in stored extracts to be absorbed onto the glass surfaces. Glass containers should be carefully precleaned as outlined in Subsection 3L in Section 3. Aluminum foil can be cleaned by agitating it in analytical reagent grade acetone followed by several rinsings with pesticide grade ethyl acetate and hexane. Plastic containers may be used, if necessary, only when non-interference with the subsequent analysis has been proven at its limit of detection. Important variables in the sampling and storage processes include the size of the sample, source, stability, contamination, intended use, behavior of the pesticides, and the temperature and time of storage.

Readers interested in a more exhaustive discussion of sampling and storage procedures than provided in the following sections of this chapter are referred to the publication "Guidelines on Sampling and Statistical

Methodologies for Ambient Pesticide Monitoring" (Monitoring Panel, Federal Working Group on Pesticide Management, Washington, DC, 1974). This 60-page manual contains chapters on statistics and study design, air, soil, the hydrologic environment, estuaries, fresh water fish, wildlife, foods and feeds, and human tissues.

6B REPRESENTATIVE VS. BIASED SAMPLING

Samples collected for the purpose of assessing tolerance infringements, such as with agricultural and food products, should be random and representative. To the contrary, most environmental samples are deliberately chosen to be biased in nature. For example, a sample of water to be analyzed for the highest possible pollution in a stream or lake would best be taken as a grab sample from the point of maximum pollution introduction (such as an effluent pipe from a factory) rather than from the center of the river where it might be most representative. If, on the other hand, the objective is an average residue profile of the entire body of water, the final sample would preferably be a composite of a number of subsamples taken at various locations and water depths. Analysis of a sick bird or fish in the middle of a metabolic cycle would usually be more useful for determining any pesticide contamination than a dead specimen which is likely to contain only metabolites. Similarly, human stomach washings (lavage) taken at an early stage are more likely to contain parent pesticides and to be useful for indication of pesticide poisoning.

It is important that the analyst be aware of these considerations and that he be consulted when deciding on the sample to be collected so that it is valid for the purpose of the analysis and valuable time is not wasted on a worthless sample.

6C SAMPLE CONTAINERS

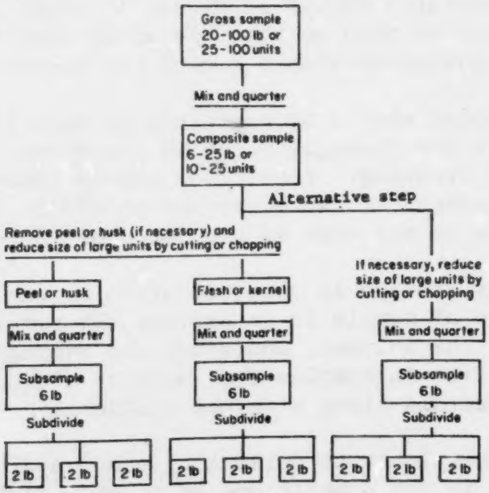
Section 2 of the EPA Pesticide Analytical Manual specifies suitable sample containers for various sample types. These include wide mouth glass bottles with foil lined screw caps for autopsy tissue samples of less than 25 grams, glass vials of at least 7 ml capacity for blood (avoid rubber or cork caps), empty pesticide-grade solvent bottles for water samples, and pint or quart capacity mason jars for larger environmental or agricultural samples. Sample collection glassware should be scrupulously cleaned as outlined in Section 3 of this Manual. Special precautions must be taken in preparing glass containers and caps and taking samples for PCP analyses because of the ubiquity of the chemical. These are outlined in Section 5,A,(4),(a),IV of the EPA PAM.

It is good procedure to clearly label collected samples with all pertinent information such as a code number, date and time of collection, type of sample, place and method of collection, description of collection site, size of sample, etc. All samples that are perishable are shipped to the laboratory in styrofoam containers with dry ice. A detailed description of systematic procedures used for receiving, numbering, and storing environmental samples at the National Monitoring and Residue Analysis Laboratory, Gulfport, MS, has been published (1).

6D SAMPLE COMPOSITING

After collection of a valid gross sample, compositing or reduction to an analytical size sample may be required, especially for agricultural and food samples. The general requirement is that the small analytical sample must be fully representative of the gross sample collected. The exact steps in the compositing procedure will depend on the particular sample involved. Figure 6-A shows typical steps in reduction of a gross sample of an agricultural product collected in the field, during processing or at the market.

FIGURE 6-A. TYPICAL STEPS IN REDUCTION OF A GROSS SAMPLE


Gross sample 20-100 lb or 25-100 units
Mix and quarter
Composite sample 6-25 lb or 10-25 units
Alternative step
Remove peel or husk (if necessary) and reduce size of large units by cutting or chopping
Peel or husk
Flesh or kernel
If necessary, reduce size of large units by cutting or chopping
Mix and quarter
Mix and quarter
Mix and quarter
Subsample 6 lb
Subsample 6 lb
Subsample 6 lb
Subdivide
Subdivide
Subdivide
2 lb
2 lb
2 lb
2 lb
2 lb
2 lb
2 lb
2 lb
2 lb

6E STORAGE OF SAMPLES

As a general rule, samples should be analyzed as soon as possible after their collection. If storage is necessary, it should be under prescribed conditions which preserve the integrity of the original sample. Samples other than water are ordinarily stored in a freezer below 0°C, but, even then, physical and chemical changes may occur in either the sample or in the residues sought. Extended storage in a freezer can cause dehydration or freeze-drying of the sample and possibly affect the subsequent analysis (2). Because many pesticides are photodegradable it is advisable to protect samples and any solutions or extracts from needless exposure to light.

Tissue samples that are to be extracted within 24 hours may be held at normal refrigerator temperature (+2 to +4°C). If extraction is not to be carried out within this time, the samples should be deep frozen at -12 to -18°C.

Blood samples that are to be separated for subsequent analysis of the serum should be centrifuged as soon as possible after drawing. If the serum is to be analyzed within a 3-day period, storage at +2 to +4°C is suitable. If storage is to be for longer periods it is preferable to deep freeze at -12 to -18°C. Otherwise, DDT may degrade in contact with broken red blood cells (hemoglobin).

Agricultural or environmental samples that are to be analyzed for organophosphates should be placed in tight containers and stored in deep freeze as soon as possible after sampling unless sample preparation is to be conducted within a very few hours.

Water samples should be extracted at once if at all possible or stored just above the freezing point to avoid rupture of the container as a result of freezing. Pesticides can be adsorbed on the glass container during storage, so the container should be rinsed with solvent if the extraction is not made in the container itself.

If lengthy storage is required prior to analysis, a good alternative to storage of sample is to extract the sample at once, remove most or all of the solvent, and store the extract at a low temperature. Decomposition in samples that must be stored can be evaluated by storing spiked controls along with the samples.

Comments pertinent to collecting samples of different types will be presented in the Subsections 6F to 6K. Methods for the analysis of the various sample types are surveyed in Section 7 of this Manual.

6F SAMPLING OF AGRICULTURAL AND FOOD PRODUCTS

Procedures for sampling, sample preparation, sample compositing, and sample reporting, as required by Federal law, for all commodity types are outlined in detail in Sections 140-143 of the FDA Pesticide Analytical Manual, Volume I. Section 3 of the Canadian Department of National Health and Welfare Analytical Manual for Pesticide Residues in Foods gives guidelines for systematically obtaining representative samples of processed and packaged foods, bulk foods, and field crops and for handling, shipping, and storing samples. Recommended minimum sizes are tabulated for different samples, with a general sample requirement of n product units, where n equals the square root of the total but need not exceed 10-15 separate units.

Section 4 of the same Canadian PAM covers laboratory preparation of analytical samples from gross samples of fresh, frozen, and canned vegetables, fruits, and juices; dry cereal grains, flakes, dehydrated fruits and vegetables; animal tissues; eggs; butter and margarine; milk and cream; cheese and nuts; fats and oil; and fish and fish products.

It is suggested that readers interested in analysis of sample substrates of this type for legal compliance to tolerance levels should refer to these two excellent sources of information. If the purpose of an analysis is to obtain information on maximum residue levels in a particular situation, biased sampling would be used, e.g., the lower perimeter of fruit would be sampled from certain trees most likely to have received a higher dose of pesticide spray.

6G SAMPLING OF BIOLOGICAL MATERIALS

Adipose tissue, blood, and urine samples from live and autopsy animal and human subjects are commonly analyzed for pesticide residues. The amounts of sample required, the time of collection, and the compound to be detected are determined by the nature of the pesticide(s) of interest. Pesticides which degrade or are metabolized readily may be absent in a particular sample, but their original presence can be deduced by determination of metabolites such as alkyl phosphates from OP pesticides, phenols from chlorophenoxy acid herbicides or carbamate insecticides, or DDA from DDT. If body tissues or fluids are analyzed quickly in cases of high exposure, the chance of finding the parent pesticide is greatly enhanced. If exposure is low or a long time has elapsed after exposure, the analyst must be familiar with pesticide metabolism in order to choose appropriate samples and metabolites to determine. For example, the highest concentration of organophosphorus pesticide urinary metabolites will be found from four to eight hours after the donor's exposure [EPA PAM, Section 6,A,2,(a),V]. When concentrations of pesticides or metabolites are expected to be small, samples must be larger, e.g., morning urine samples or 24-hour pooled specimens.

The majority of human adipose tissue samples are taken during autopsy by an attending physician. Samples should be placed in a clean glass container with a foil-lined screw cap. Plastic bags must be avoided since they can contribute traces of impurities such as phthalates to the sample, causing spurious GC peaks when the final concentrate is examined by EC-GC [EPA PAM, Section 5,A,(1),V].

Whole blood samples are transferred to glass vials with Teflon or foil lined screw caps, and the required serum aliquot is removed after a period of settling in a refrigerator and subsequent centrifugation. Serum is stored in a refrigerator at 2-5°C if the analysis is to be performed in 24 hours or in a deep freeze (-15 to -25°C) for longer periods. The analysis of chlorinated pesticides is not adversely affected by such storage for periods up to one month [EPA PAM, Section 5,A,(2),(a),IV].

6H AIR SAMPLING

The EPA has in the past operated a nationwide air monitoring program in order to gather information on the extent of human exposure to airborne pesticides. This program utilized Greenburgh-Smith impingers containing ethylene glycol for trapping organophosphorus and halogenated hydrocarbon insecticides both in the vapor phase and as dusts. The air was drawn through the impingers by means of a vacuum pump, the amount sampled (m^3) being controlled by means of a flow meter and timer.

The usual sample consisted of 400 ml ethylene glycol, representing the contents of four impingers containing 100 ml each. Two impingers were operated simultaneously for 12 hours, then the other two for an additional 12 hours. The total air volume sampled in a 24 hour period was generally ca. 80 m^3. The ethylene glycol samples and the filters used in the machine to collect particulate matter were shipped to the laboratory in labeled glass containers. Refrigeration of the collected air samples was unnecessary except in cases of long delay between sampling and analysis.

The ethylene glycol sampling procedure has not proven to be wholly acceptable in terms of convenience or reliability. Although ethylene glycol is an efficient medium for dissolving pesticide residues, it is difficult to obtain glycol of adequate purity to reduce background in the subsequent analysis. The EPA national air sampling program has been discontinued as of this writing pending the development of sampling and analysis procedures of higher volumes of air than has been possible in the past. However, the ethylene glycol impinger continues to be used by some laboratories in local monitoring programs (1, 3, 4).

Research (5) in the EPA methods development laboratories has indicated that a small cartridge packed with ca. 1 gram Chromosorb 101 adsorbent allows analysis of chlorinated hydrocarbons in air down to 15 ppt after 2 hours of sampling at a rate of 1 to 2 liters/minute. The compounds are efficiently trapped on these small plugs, which can be very conveniently transported to the laboratory for analysis. The short path length of the collector is possible because of the low air volume used. Chromosorb 101 was used uncoated after purification by extraction and activation by heating. Collected pesticides were eluted from the adsorbent with an appropriate solvent (e.g., hexane for HCB) prior to analysis.

Other proposed media for collecting chlorinated and phosphorus pesticides and other organic pollutants in air include glass beads coated with cottonseed oil in a high-rate sampler (6), support-bonded chromatographic phases such as 24% $(C_{18}H_{37}SiO_{3/2})_n$ on Chromosorb A or 31% $(C_{18}H_{37}SiO_{3/2})_n$ on Diatom W (7), and columns of Tenax GC and carbon adsorbents (8). Adsorption and desorption (at 250°C) of biphenyl at greater than 90 percent efficiency was demonstrated with Tenax (9). 2,4-D acid and its ester and amide derivatives were trapped from air and recovered with 86-96 percent efficiency using tubes containing XAD-2 resin (10).

Another approach is exposure of a nylon chiffon cloth screen, saturated with 10 percent ethylene glycol in acetone, to the atmosphere for a number of days, followed by Soxhlet extraction of the pesticides from the cloth with hexane-acetone (1:1 v/v) (11, 12). Results with the screen are only qualitative, as the amount of air passing through is not rigorously measured. A similar method involved adsorption on layers of polyethylene glycol 400 spread on stainless steel nets followed by extraction of the pesticides into petroleum ether or benzene before GC determination. Inclusion of glass fiber filters plus coated nets in the collection tubes differentiated aerosol-bound pesticides and vapor fractions (13). Composite filters composed of layered glass fiber and Porapak R have been shown to efficiently collect malathion (13a), methyl parathion (13b), and chlordane/heptachlor (13c) at flow rates of 150 to 170 liters/minute. The carbamate insecticide propoxur (Baygon) was collected from air in a Greenburgh-Smith impinger containing NaOH. The solution was acidified and the pesticide extracted with benzene prior to analysis (14). Hexylene glycol contained in glass scrubbers has been used to recover dieldrin and heptachlor from air at 0.1 ng/m^3 (15).

One of the most promising approaches to the sampling of air involves use of polyurethane foam. The updated review of air sampling methods in Section 8,A of the EPA PAM contains a discussion of this method in addition to other approaches. Polyurethane foam vapor traps following a particle filter have been evaluated (16) for sampling of pesticides, PCBs and polychlorinated naphthalenes. Collection rates up to 360 m^3 of air per 24 hours and sensitivities as low as 0.1 ng/m^3 for some compounds were achieved. The filters and plugs were Soxhlet extracted with hexane-ethyl ether (95:5 v/v) at 4 cycles per hour for 16-24 hours, and OCl pesticides were determined by EC-GC after alumina column cleanup and OP pesticides by FPD-GC without cleanup. Collection was generally satisfactory but was poor for the more-volatile OCl compounds. Recovery was ca. 75 percent for OP pesticides. It was shown that a second trap in series with the first did not necessarily improve recovery values. The collection of dieldrin, lindane, trifluralin, dacthal, chlordane, and heptachlor on polyurethane foam was studied and an optimization method given for the plug size and shape for any chosen sampling rate. Trapping efficiency depended upon pesticide vapor pressure and the flow rate of air. The quantitation limit was ca. 0.1 ng/m^3 in a 5 m^3 air sample. It was crucial that the plugs were carefully protected from laboratory contamination and Soxhlet extracted with pesticide-grade acetone and hexane prior to use if clean blanks and highest sensitivity were to be achieved (17).

Because of possible pesticide degradation, air sampling apparatus should be shielded from light during sample collection.

6I WATER SAMPLING

The design of a comprehensive pesticide sampling program for environmental waters is a specialized topic which is covered in publications available from the Water Quality Control Division of the USEPA, National Environmental Research Center, Cincinnati, Ohio. Important considerations include the objective of the study, frequency of sampling, location of sampling stations as related to hydrologic conditions, and the selection of sampling methods. The following is a brief review of some important selected factors in a sampling program.

a. Grab Samples

Water can be collected by taking one instantaneous ("grab") sample from a given location, directly filling the sample container. The usual technique is to submerge the container a few inches below the water surface during filling to avoid skimming off any floating film which would be least representative of the vertical water column. Several collections should be taken at various depths and locations to provide a more representative sample. Care should be exercised to avoid disturbing bottom sediment. Discrete samples from various depths can be obtained with standard samplers consisting of a metal outer container with a glass sample collection bottle inside (e.g., Precision and Esmarch samplers, EPA PAM, Section 10, A, II). Grab sampling is often sufficient for lakes, reservoirs, etc., that are not subject to rapid transitional changes.

Grab samples less than 2 liters are collected in wide mouth glass bottles, and samples of one gallon or more in the glass bottles in which pesticide quality solvents are supplied. All bottle caps should be Teflon lined. The sample size is dictated primarily by the expected residue levels, the sensitivity of the analysis, and the need to run duplicate, spiked, and background analyses. A 500-1000 ml sample may suffice from water where pesticide levels are expectedly high, while two liters or more may be needed for a surveillance program where no high levels are anticipated. Rainwater is collected in clean glass containers rather than metal or plastic. Samples should include information that will help the analyst choose a proper analytical method and interpret the results. This includes the location of sampling, depth, suspected contaminants, type of sample (surface water, waste discharge, etc.), and agricultural activity or spills in the immediate area or upstream.

Many pesticides are unstable in water, so samples should be analyzed as soon as possible after collection, ideally within a couple of hours. If this is impractical because of distance from the sampling site to the laboratory and/or the laboratory work load, storage should be made in a refrigerator or freezer. Samples being examined solely for organochlorine residues may be held up to a week under refrigeration at 2 to 4°C with no adverse effect. Those samples to be analyzed for organophosphorus or carbamate pesticides should be frozen immediately after drawing the sample because of rapid degradation in aqueous media (Table 1, Section 10,A of the EPA PAM shows data for the degradation rate of 29 pesticides in water at ambient temperature in sealed containers (18). pH adjustment is required for some samples immediately after collection (e.g., adjustment to pH 2 with sulfuric acid for phenoxy acid herbicides). Holding time and storage conditions must be reported along with the analytical results and corrections made if rates of pesticide degradation are known. Exposure of samples to sunlight should be avoided.

Every effort should be made to perform the solvent extraction step at the earliest possible time after sampling, irrespective of the classes of pesticides suspected of being present. Especially unstable pesticides can be extracted immediately in the field. The resulting extracts can be safely stored for periods up to three or four weeks at -15 to -20°C before proceeding with subsequent cleanup and determinative steps. One disadvantage of glass sample bottles is possible breakage in shipment, and care should be exercised in proper packaging to avoid this. Another disadvantage is the already-mentioned possibility of pesticide adsorption on glass surfaces. Reduced recovery (>90 percent to 46-68 percent) of DDT in water analysis upon storage has also been noted due to adsorption on suspended matter in the sample (19).

The assumption made is that a grab sample is at least representative of the immediate water mass from which it was taken and, hopefully, somewhat representative of the water that will pass the sampling point during some limited future time interval. The grab sample is amenable to use in both random and nonrandom sampling programs. The number, frequency, and distribution of samples collected will depend upon the study objectives and the variability within the "population" being sampled.

After sampling, pesticides are extracted from water, cleaned-up and concentrated as necessary, and determined by GC or an alternate method. Pesticides in clean water (e.g., drinking water) can be detected at 5-500 ppt levels by electron capture GC without the need for extensive extract cleanup. Impurities in "dirty" samples will require additional cleanup steps, and background problems will cause difficulty in analyzing these low levels accurately.

b. Continuous Samplers

Continuous and automatic devices are often used for sampling flowing bodies of water such as rivers and streams. Activated carbon filters have been widely used for adsorption of pesticides and other kinds of organics in natural waters since they were developed and introduced by the U.S. Public Health Service in 1951 (20). The technique involves passage of a continuous, constantly controlled volume of water through a column of activated carbon followed by desorption by means of elution or by Soxhlet extraction with a suitable solvent or combination of solvents. The variable efficiency and consistency of pesticide adsorption and desorption from the adsorbent prior to determination, ease of contamination with extraneous organic substances, and bacterial and oxidizing attack on the sorbed pesticides have caused problems with carbon columns (21, 22).

Filter matérials which have been recommended as alternatives to carbon for collection of pesticides (usually chlorinated insecticides) from natural waters include reversed liquid-liquid partition systems (a hydrophobic phase coated on a support) and other adsorbents. Carbowax 4000 (5 g) and n-undecane (23), silicones chemically bonded to diatomaceous earth support (24), covalently bonded aromatic and alkyl chlorosilanes on Celite (25), porous polyurethane foam columns (for pesticides and PCBs) (26, 27), polyethylene film (20-25 μm thickness) (28), and polyurethane foam coated with selective adsorbents (29) have all been used with varying success.

The XAD macroreticular adsorbent resins (XAD-1, -2, -4, and -7) have been used to collect organics from both potable (30, 31) and sea (32) water. Optimum conditions for use with XAD-4 resin were found to be 2 grams of adsorbent, a flow rate through the resin of 8 ml/minute, and 100 ml hexane-diethyl ether (10:1 v/v) as eluting solvent. Among 10 chlorinated insecticides studied, only aldrin and *p,p'*-DDE were not quantitatively recovered, and recovery of PCBs was 76 percent (33). Details for use of XAD-2 and -4 resins for many classes of trace organic water contaminants have been published (34) and recoveries between 81 and 96 percent reported for 20 ppt levels of atrazine, lindane, dieldrin, DDT, and DDE (47 percent for aldrin). An EPA report (35) recommends XAD-2 resin for routine monitoring of sea water for chlorinated insecticides and PCBs. Average recovery for XAD-2 extraction of fortified natural waters collected across Canada was 85 percent for the 10-100 ng/liter levels of 10 OCl pesticides (recovery of mirex was unacceptably low) and 82 percent for 250 ng/liter levels of PCBs; blanks from the resin were a low 4 ng PCBs/liter (36). Amberlite XAD-4, porous polyurethane foam, and undecane plus Carbowax 4000 on Chromosorb were found to be comparable for extracting 10 OCl insecticides from environmental water samples (23).

Continuous liquid-liquid extractors are an alternative to the filter-adsorbent processes preferred by some analysts. A multichamber extractor with internal solvent renewal replenishing (37) allowed extraction of 135 liters of water at rates of 0.5-1.0 liter/hour and recovered greater than 97 percent of ppb levels of pesticides. Subsequently, a similar modified apparatus permitted use of both heavier-and lighter-than-water solvents (38). A simple and rugged field version of the Kahn and Wayman apparatus (37) excluded solvent recycling and was based on mixed settling (39). This apparatus, which consisted of an extraction unit, magnetic stirrer, and pump, provided quantitative recovery of pesticides and PCBs at levels of 0.1-1.0 ng/liter of river water.

More recently, a similar *in situ* apparatus designed to solvent-extract large amounts of sea and river water continuously while situated at a desired depth at the sampling site has been described (40). A Teflon helix, continuous liquid-liquid extractor, plus a continuous evaporative concentrator recovered μg to ng per liter amounts of OP pesticides from river and sea water or from secondary sewage effluent with >80 percent efficiency (41). A comparative study of recoveries from river water by continuous extraction and activated carbon filters showed that the recoveries were similar but the former was less costly (42).

The theory for extracting chlorinated pesticides continuously from water with a stationary immiscible solvent is discussed in reference (39).

See Section 10,A,III-V of the EPA PAM for updated material on water sampling.

6J SAMPLING OF HOUSE DUST, SOIL, AND STREAM BOTTOM SEDIMENT

House dust is collected with a vacuum cleaner, air dried, and sieved prior to analysis. Soil is sampled by collecting cores or borings of a known diameter cut to a depth of ca. 3-4 inches or more from the centers of plots one square meter in size. Ten to twenty cores representing a surface area of at least 200 cm^2 are recommended. The first two inches of core, containing the grass or crop cover and roots, are separated from the underlying soil. Corings representing each layer of soil are combined, quartered, and divided into 2 lb samples for analysis. Soils are analyzed in an air-dry state after sieving to remove foreign material. Another reported procedure for soil sampling (1) involves collection of cores 1-3 inches deep and 3 inches in diameter with a hand-operated auger; on a 1/4 acre site (105 feet x 105 feet), sampling begins 7.5 feet from the border of the site, and a core is collected every 15 feet until 7 cores are obtained. The process is repeated along parallel lines separated by fifteen feet from the original sampling line, until a total of 49 cores is collected. The cores are sieved through a hardware cloth screen into a 3 gallon galvanized pail and thoroughly mixed. The sample is transferred to two one-half gallon cans with lids for shipment to the laboratory. There is no way to collect a truly representative soil sample, and reproducibility of results on different samples taken from the same area is often expectedly poor.

Sediment from the bottom of a body of water provides information concerning the degree of pollution resulting from pesticides, particularly those that are not readily degradable. This information combined with residue data on the water and resident biological life gives an overall pesticide contamination profile of the body of water. Bottom sediment varies with respect to both particle size composition (surface adsorptive power) and organic content. Therefore, sample sites should be selected at random in an effort to collect samples representing a range of variation. In some cases consultation with an oceanographer can indicate where one would be likely to find the maximum amounts of pollution from considerations such as currents and industrial effluent discharges.

About a quart of sediment is a typical sample size. Actual collection is accomplished with one of a variety of core samplers or dredges. A diagram of a dredge-type device for collecting sediment samples has been published (1). The dredge is thrown into the water at least 10 times to collect samples, which are transferred each time to a galvanized pail. The total sample is mixed and transferred to one-half gallon cans (with a hole in each lid to release any gas buildup from organic matter in the sample) for shipment to the laboratory. A simple bottom sediment collector composed of a steel can attached to the end of an aluminum pole has also been described (43). Samples may be preserved with formalin or a variety of other sterilants provided they do not affect the analyses to be run. Samples are air dried and ground prior to analysis. They are stored, if necessary, in a freezer if volatile compounds such as 2,4-D ester may be present.

6K MARINE BIOLOGICAL SAMPLES AND OTHER MISCELLANEOUS SAMPLES

A problem sometimed encountered when collecting plankton and bottom organisms is to obtain the minimum weight necessary for successful analysis. As a general rule, a minimum of about 10 grams will be required. Collected organisms can be frozen at once or preserved with 5-10 percent formalin or 70 percent ethanol, prepared with distilled water rather than the water from which the collection was made. This eliminates the possibility of pesticides in the water concentrating in the organisms over a period of time. Any added preservative must be extracted and analyzed to determine if exchange of pesticides from the organisms to the preservative has occurred.

Sufficient masses of plankton are collected by use of a tow net behind a boat or by pumping water through a net. Bottom fauna are collected with dredges or dip nets. Samples are washed through a screen and organisms hand picked from the remaining debris.

Fish are collected utilizing seines, gill nets. traps, electrocution devices, otter trawls, or angling. Wrapping the fish in aluminum foil and preservation by quick freezing in dry ice is most desirable. When this is not possible, liquid preservatives are used. Larger fish should be injected with preservative from a syringe to prevent decomposition of internal organs. Fish stored in formalin plus 5 percent $Na_2S_2O_3$ showed no loss of Abate (temephos) residues (>1 ppb) for up to three weeks (44). Fish can be analyzed whole to yield data on gross contamination, or the fish can be sectioned to obtain information on edible and non-edible parts. Analyses of individual organs and tissues yield information on distribution of pesticides in the fish. Analysis of blood from a dying fish may be valuable for determining probable cause of death where pesticide exposure is suspected. The blood is obtained by cutting off the tail at the caudal peduncle and collecting and freezing the blood in a small vial.

Invertebrate samples are collected in pitfall traps, as described by Wojick et al. (45). Bird samples are collected using Japanese mist nets placed near a water source or in a cove where the net is not visible. Traps baited with peanut butter or some other foodstuff are employed for sampling mammals. These traps, which are available in a variety of sizes, have a trap door that closes when the animal enters to take the food. Non-crop vegetation samples are obtained with shears, sickles, pocket knives, etc., usually from the same sampling area as soil samples. All of these samples are sorted, wrapped in aluminum foil with the shiny side out, tagged, and placed in a plastic bag for shipping (1).

Some of the material in the sections on sampling was adapted from an EPA training course manual (46). A review that includes some of the above sampling procedures and additional methods for collection of environmental samples has been published (1).

6L CONTROL OF PROCEDURES FOR EXTRACTION OF RESIDUES

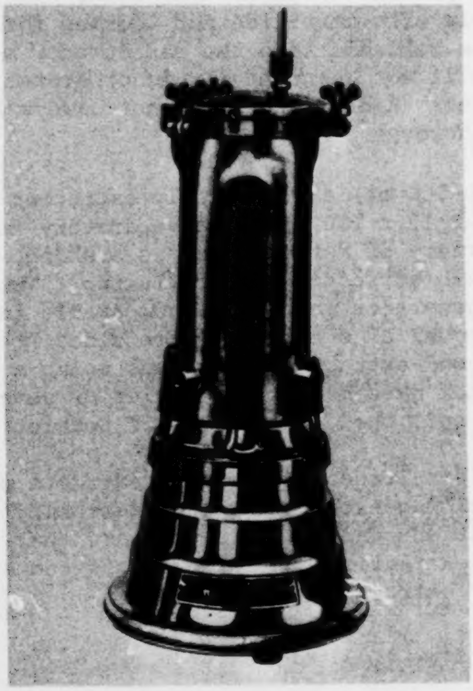
Specific procedures for the extraction and cleanup of pesticide multiresidues in many sample types are surveyed in Section 7 of this Manual. This subsection discusses general considerations of pesticide extraction from collected samples.

Many solvents are employed for extracting residues, depending upon the polarity of the pesticide and the amount of co-extractives expected from the particular substrate. Solvents range from hexane or petroleum ether for nonpolar organochlorine and organophosphorus compounds to methylene chloride (dichloromethane) for polar carbamates. Chloroform, diethyl ether, ethyl acetate, benzene, acetonitrile, methanol, acetone, and various two- and three-component mixtures of these have all been widely used. Addition of acid to the organic solvents may aid extraction of acidic pesticides such as 2,4-D herbicide. Acetonitrile is an excellent general purpose extraction solvent for low fat-content samples (acetonitrile plus ca. 35 percent water for low moisture samples), and hexane/acetonitrile systems are widely recommended for partition cleanup.

Different techniques are employed for bringing the extraction solvent and sample into contact. The best extraction is obtained, in general, by achieving the most intimate contact between the two, although the type of residue is an important distinction. When emulsions result from vigorous shaking or mixing during extraction procedures, centrifugation will usually be effective in separating solvent layers. A surface residue can usually be extracted by a simple washing procedure, while the more common internal residues can be extracted only after fine masceration of the sample. Some soil samples tenaciously bind pesticides and require long periods (e.g., 8 hours) of Soxhlet extraction rather than shorter periods of blending as is common with plant materials.

Blending of the sample plus solvent in a Waring Blendor (Figure 6-B), omni-mixer, or Wiley mill is probably the most usual extraction procedure in use today, especially for biological, plant, and food samples. Some additional sample subdivision, such as cutting, chopping, or grinding usually precedes the blending operation. A five minute period of blending at a moderate speed is typical for many samples. A special device for aiding formation of a homogenous sample has been described (47). The device, consisting of a handle and shaped aluminum sheet, fits inside a blender jar and serves to gently push bulky samples into the cutting blades during the blending operation. A liquid-nitrogen cooled freeze grinder for biological materials containing labile pesticides has also been devised (48).

Figure 6-B. Waring Aseptic Dispersall Model AS-1. (Shown on 702-CR Base)



Blending with a solvent followed by filtering or centrifuging is particularly efficient for most vegetable samples. The water in the sample may give rise to emulsions with nonpolar solvents, and this can often be avoided by use of a drying agent such as anhydrous Na_2SO_4 or 2-propanol together with, or before, the solvent. Meat samples containing too much connective tissue for a blender to deal with effectively should be first comminuted by a grinder. Simple heating of minced sample in a beaker on a steam bath with solvent can be effective, possibly after grinding the sample with Na_2SO_4 and sharp sand to help break down some connective tissue. More volatile pesticides (e.g., lindane) might be lost in this way.

In some cases, more exhaustive extraction of residues from difficult samples can be obtained by Soxhlet extraction for periods up to 12 hours or longer with a solvent such as methanol-chloroform (1:1 v/v) (49). Preliminary steps such as drying, grinding, or chopping normally precede the extraction. (Soxhlet thimbles may require exhaustive extraction prior to use so they do not contribute interferences to the analysis (50)). Even this procedure may not give complete extraction in all cases, and only studies with samples to which radioactive tracers have been applied can indicate the absolute extraction efficiency in any particular case. The usual evaluation procedure of spiking a sample with pesticide and looking for quantitative extraction is less reliable than the radiotracer method because the spiked chemical will not be naturally incorporated in the same matrix as would the tracer. Radiotracers are not always available or feasible to use, however.

Water samples (100-500 ml) are generally extracted by shaking with an appropriate solvent (3 x 100 ml) in a separatory funnel. Soils are extracted by a variety of methods such as shaking, soaking, blending, Soxhlet or Goldfisch extraction, or refluxing. Two 15 minute extractions in an ultrasonic generator were found comparable to a 24 hour Soxhlet extraction for removal of s-triazine herbicides from fortified soils [51], and a 30 second extraction technique using a Brinkmann Polytron ultrasonic generator gave better recoveries of several chlorinated insecticides from soil than did 8 hours of Soxhlet extraction [52].

An apparatus that simultaneously Soxhlet extracts pesticides and concentrates the resulting extract has been designed [53]. Advantages of this cyclic extraction-evaporation system are that distillation of solvents prior to extraction can often be omitted and excess solvent is re-utilized for extraction.

The most efficient solvent and parameters for extraction of pesticides from water can be determined using the p-values originally suggested by Beroza and co-workers for use in residue confirmation (Subsection 8F in Section 8). The p-value is the fraction of total pesticide that is distributed into the nonpolar phase of an equivolume immiscible pair of solvents. This approach was used to study the extraction of OP pesticides from water (54), and the best solvents were found to be benzene, ethyl acetate, or diethyl ether for diazinon and diazoxon at pH 7.4, ethyl acetate for malathion at pH 6, and diethyl ether or ethyl acetate for fenthion (Baytex) at pH 3.4. p-Values can also be used to theoretically select water-to-solvent ratios and the optimum number of extractions for maximum recovery of a pesticide in water (55). Using 2,4-D as a model, it was shown that a pesticide with a p-value equal or greater than 0.90 can be 95 percent extracted from 1000 ml aqueous phase by up to five extractions of ≥50 ml with a total volume of solvent up to 500 ml. The best solvents and conditions were then worked out on a practical scale for extraction of 2,4-D and 2,4,5-T and their esters (56). Diethyl ether or ethyl acetate was found best for 2,4-D acid and esters and benzene for 2,4,5-T acid and esters, and a 99 percent recovery of 2,4-D from one liter of aqueous solution was obtained by a two stage serial extraction with 200 ml and 50 ml of ethyl acetate under conditions predicted by p-values.

6M CONTROL OF METHODOLOGY FOR CONCENTRATION OF SAMPLE SOLUTIONS AND FRACTIONAL ELUATES

The concentration of cleaned-up sample in the injection or spotting solution is one important factor that determines if sufficient residue is available for detection by GC, LC, or TLC. The analyst must determine this and concentrate final solutions according to the least sensitive pesticide in the method's scope.

Purified extracts or eluate solutions are concentrated to a volume of ca. 5 ml using a Kuderna-Danish evaporative concentrator flask fitted on top with a 3-ball Snyder reflux column and a collection tube on the bottom (Figure 6-C).

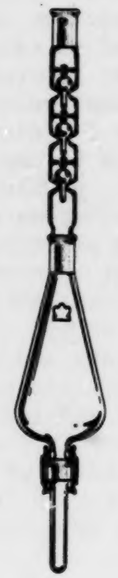


Figure 6-C. Kuderna-Danish Evaporative Concentrator, Kontes Glass Co. No. K-570000.

The tube is heated in steam water bath in a hood. The apparatus should be mounted or held so the lower rounded flask surface is bathed in steam. Flasks, which range in size from 125-1000 ml, should be initially charged with 40-60 percent of their nominal volume, and the column should be pre-wet with ca. 1 ml of solvent before beginning concentration to prevent possible initial small loss of pesticides. Refluxing is continued until the final concentrate is collected in the lower tube. Boiling chips are required for smooth operation of the K-D evaporator, and carborundum, checked for absence of contamination, is recommended in preference to porcelain, vanadium, or glass chips.

For concentration from 5 ml to smaller volumes (as low as 0.1-0.5 ml), the concentrate is cooled, the collection tube is removed from the K-D flask, and a fresh chip is added. A micro-Snyder reflux column (Figure 6-D) is fitted directly to top of the tube and evaporation is begun by holding the bottom of the tube in a steam or hot water bath. Evaporation is continued, with care to avoid bumping, to slightly below the desired volume. The tube is withdrawn from the water when boiling agitation becomes too vigorous; immersion and withdrawal are alternated based on observation of boil agitation. The apparatus is cooled 3-5 minutes, and condensate is allowed to drain down into the tube before removing the column. The sides of the tube and column joint are rinsed with solvent to avoid hang-up of pesticides on upper glass surfaces. A 1-2 ml syringe is useful for performing this rinse. Finally, further fresh solvent is added to dilute up to the desired volume, if necessary

A special rack which simultaneously agitates and evaporates solutions in six concentrator tubes fitted with micro-Snyder columns in a time equal to a single tube is described in the EPA PAM, Section 5,A,3,a.

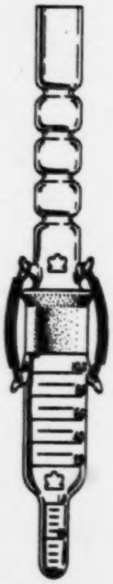


Figure 6-D. Semi-Micro Kuderna Danish Apparatus, Kontes Glass Co., No. K-569250.

Extracts containing fats, oils, or plant extractives, or purified extracts to which "keeper solution" has been added, can be evaporated on a rotating vacuum type evaporator with the water bath at, or just slightly above, room temperature. Extracts contained in a beaker or a centrifuge tube immersed in a water bath at 40°C can be evaporated under a stream of nitrogen adjusted to cause gentle depression on the surface of the solution. The nitrogen should be passed through well maintained scrubber tubes to remove contaminants which could cause pesticide degradation. Warming a tube by holding it in the hand is a useful, gentle evaporation aid during nitrogen blow-down.

An evaporation assembly combining an evaporative concentrator tube, a Kuderna-Danish flask, and a rotary vacuum evaporator (Figure 6-G) is shown in Section 10,A of the EPA PAM, Figure 1. The concentrator tube is not immersed in a high temperature water bath as usual, but rather in a 35°C water bath to minimize degradation of heat labile pesticides. This apparatus confines the concentrated extract to one container, thereby eliminating the need for transfer. One hundred ml of methylene chloride can be reduced to 5 ml in ca. 20 minutes with a vacuum of 125 mm of mercury.

An excellent multitube apparatus for nitrogen evaporation is available from Kontes Glass Co. (57). Concentration is rapid until the solution reaches 0.5-1.0 ml, at which point evaporation slows markedly because this last volume is below the heating zone of the evaporator block. Thus, losses of pesticides from inadvertent evaporation to dryness (58) are avoided, and a minimum of analyst attention is required (Fig. 6-E).

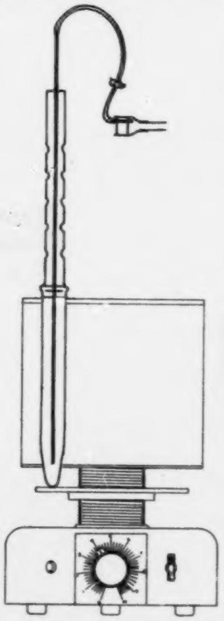


Figure 6-E. Ebullator, Kontes Glass Co.

A distillation column is fixed on top of the tube holding the sample, and small bore stainless steel needlestock tubing is fitted through the column down into the tip of the tube to direct a stream of micro bubbles of nitrogen through the solution to initiate and maintain ebullation. Recoveries of seven chlorinated pesticides after concentration for two hours in this apparatus were greater than 94 percent with both hexane and benzene solvents.

It is important to avoid pesticide loss or decomposition during evaporation steps. Numerous reports have been made [e.g., 58, 59] of severe pesticide loss during concentration steps, even in the presence of sample coextractives. There was no correlation between the amount of coextractives and evaporative losses, but apparently the nature of the coextractives may be important. In most situations, organochlorine and organophosphorus pesticides can be concentrated to small volumes without loss by the K-D evaporative procedures described at the beginning of this subsection. Evaporation to dryness should never occur. If the complete removal of a particular solvent is required, solvent exchange can be carried out so that the sample never gets to dryness. For example, hexane can be completely removed by boiling-down to a low volume and adding small volumes of acetone as evaporation continues until all hexane is eliminated.

The use of air for concentration of an extract should best be avoided. Satisfactory recoveries are obtainable when the residue levels are relatively high, but significant losses have been documented of even the more stable pesticides at low concentration levels [58].

A commercial tube heater which avoids evaporation to dryness with micro K-D apparatus was originally described by Beroza and Bowman [60] (Figure 6-F). Six extended-tip K-D concentrator tubes are accomodated and simultaneous evaporation to less than 1 ml can be carried out without attention.

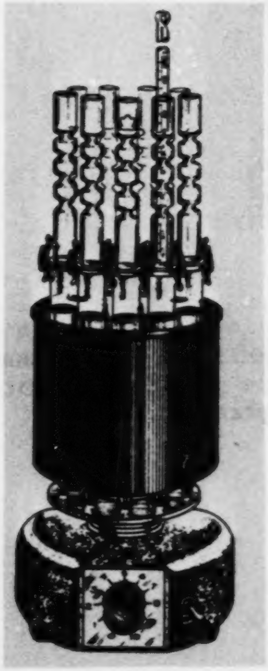


Figure 6-F. Tube Heater, Kontes Glass Co., K-720000

Other reports of pesticide loss include dieldrin and DDT when an extract was evaporated in the presence of light [61] and carbamate pesticides when evaporated in a K-D apparatus [62]. In the latter case, rotary vacuum evaporation (Figure 6-G) at 50-55°C with addition of a keeper solution was recommended. Many carbamates can be successfully evaporated under a nitrogen stream without loss after adding a keeper. A satisfactory general purpose keeper is 5 drops of 1 percent paraffin oil in hexane. Solutions containing the herbicide Balan (benefin) cannot be evaporated in a current of air without loss of pesticide, whereas rotary evaporation at a temperature of 50°C or less is successful. All evaporation and concentration steps should be checked with spiked samples if any question of pesticide loss should arise.

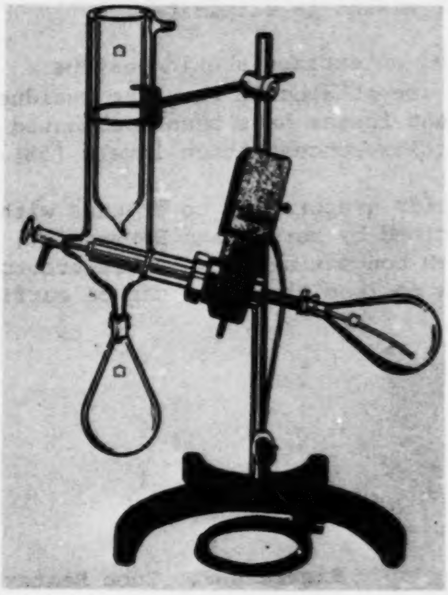


Figure 6-G. Rotary Evaporator, Kontes Glass Co., No. K-570160

The importance of clean glassware in all parts of a pesticide analysis has been stressed several times earlier in this Manual. The special importance of clean glassware to be used for concentration of solutions to small volumes cannot be overemphasized.

The final solution to be used for the determinative step must be composed of a solvent appropriate for the particular analytical procedure. Choice of a volatile solvent for partition and column cleanup procedures is advantageous because evaporation to an appropriate volume can be carried out quickly enough to be practical. If a different solvent is required for the final sample solution, solvent exchange can be carried out by taking up the nearly dry residue in the new solvent after evaporation. Solvents for GC and LC are restricted by the selectivity of the detector, while for TLC almost any volatile solvent is useful for the solution to be spotted. Chlorinated solvents cannot be present in the injected solution when an EC or the Cl modes of the MC or electrolytic detectors are to be used. Acetonitrile has an adverse effect on the response of the EC detector, while aromatic and halogenated compounds and acetonitrile increase the response of the thermionic detector. The most volatile solvent possible should be used to shorten the venting period and minimize loss of early eluting pesticides for those detectors that require solvent venting (e.g., FPD and CCD). A solvent free of UV absorption is required for the detection by the ultraviolet LC monitor.

HIGH PERFORMANCE LIQUID COLUMN CHROMATOGRAPHY (HPLC)

6N INTRODUCTION TO HPLC

High performance liquid chromatography is becoming increasingly important as a powerful technique for the separation and analysis of pesticide residues. HPLC is a very gentle technique which commonly operates at ambient temperature. It has advantages over gas chromatography where compounds are not naturally volatile and derivatization is difficult or unsatisfactory, and for thermally labile compounds. Sample preparation may be much simpler for LC since complicated matrices can often be injected directly where GC would require a prior cleanup procedure. Compounds which may not gas chromatograph well unless derivatized (e.g., phenoxy acid herbicides, which require methylation prior to GC) often give excellent liquid chromatograms [63]. The two most widely used detectors in LC, the differential refractometer and ultraviolet (UV) photometer, are nondestructive to the sample, making fraction collection a routine matter. LC is ideally suited to sample scale-up, and because of high loadability, detection of the sample is usually not a problem. The separated compounds emerge from the chromatograph dissolved in solvent which can easily be collected and the solvent removed to recover the compound. A greater variety of separations of more complexity can be achieved by LC because of the active role played by the mobile phase as contrasted to carrier gas in GC. HPLC has been used for cleanup of pesticide extracts prior to GC determination [64] as well as for the final determination itself.

Disadvantages of LC are that detector sensitivity is not comparable to that obtainable with GC detectors, especially electron capture, and a wide range of element selective detectors is not yet available. In general, present commercial LC detectors have sensitivities in the 10^{-5} to 10^{-9} gram range. In one comparative study of GC and HPLC (63), detection limits were 100-1000 times better for DDT and 2,4,-D than with an LC photometric detector, 5 ng of each pesticide being detected with the latter at 210 and 278 nm, respectively. However, the poorer sensitivity could be overcome by injection of large sample volumes (e.g., 50-100 μl) without loss of linearity or peak symmetry. The ability to introduce large volumes in LC can make sensitivity between the two methods comparable. Derivatization methods can increase the sensitivity of detection, e.g., by formation of UV-absorbing or fluorescent derivatives, but only at the cost of more complicated sample preparation. HPLC is still relatively new, and as improved equipment is developed and analysts obtain a better knowledge of HPLC, this technique will be taking its place beside GC and TLC as an important tool for pesticide residue determinations.

60 HPLC Instruments

The basic elements of a complete, automated HPLC instrument include a solvent reservoir and gradient forming device, high pressure pump, injection system, column, detector, and recorder (Figure 6-H). The instrument components must be joined by tubing that is as short and as narrow in bore as possible with low dead-volume fittings and valves so as to minimize extra-column peak spreading. The gradient device mixes together various solvents to produce a continuous or stepwise change in chemical composition (e.g., polarity or pH) of the mobile phase during the elution. Gradient elution is analogous to temperature programming in GC since both are used to speed and optimize complex separations. In adsorption and partition LC, the gradient usually involves an increase in solvent polarity; in reversed phase partition LC, solvent polarity is progressively decreased.

Figure 6-H. High performance liquid chromatograph including two Model 6000 pumps, a Model 660 solvent programmer, and Model 440 UV absorbance and R401 differential refractometer detectors. Waters Associates.

The pumping system must provide the pressure required to achieve the correct flow rate through the column. Although most instruments permit pressures up to at least 5000 psi, the vast majority of analytical separations can be done at pressures ranging from a few hundred psi to about 1200 psi. HPLC pumps fall into two categories, namely, continuous displacement (e.g., gas displacement, gas amplifier, and syringe types) and intermittent displacement (e.g., peristaltic, diaphragm, and reciprocating piston). The continuous-displacement pumps deliver a smooth, pulseless flow of solvent but have a limited solvent reservoir, which may require interrupting a run for refilling. They are inconvenient for gradient elution because the composition of solvent in the pump cannot be changed during the run, and the solvent gradients must be formed at high pressure downstream from the pump. Intermittent displacement pumps operate from an open, unlimited solvent reservoir at ambient pressure, and solvent refill or formation of solvent gradients upstream at low pressure is no problem. These pumps have a pulsating output which must be damped, electronically filtered, or cancelled-out by a two pump head arrangement.

Injection is carried out in one of three ways in different commercial LC instruments. Stop-flow injection involves shutting off the flow of solvent in the columns (either by stopping the pump or by using a shut-off valve), removing a cap from the head of the column, and injecting the sample directly on top of the column. Because diffusion in the liquid mobile phase is negligible compared to gaseous diffusion in GC, the cap can be resealed and chromatography resumed without significant loss of efficiency. Two different injector systems allow sample introduction from a syringe without stopping solvent flow. With a septum injector, the sample is injected directly into the flowing solvent with a technique similar to that used in GC. These injectors are most suitable for work at lower pressures. Septum deterioration from solvent attack and coring of septa during injection against high pressure are common problems with this type of system. With the septumless injector, the sample is introduced at atmospheric pressure into a loading loop that is being bypassed by the pump-to-column stream; by switching to the "inject position" the loop becomes part of the main solvent stream and the sample is immediately swept onto the column. The third type of injection system is a rotary injection valve in which an external loop is completely filled with sample, and the loop is then inserted into the flowing stream. It differs from the septumless syringe injector because the precise loop volume determines the amount of sample introduced rather than a syringe. A number of automatic devices for sequential introduction of multiple samples is also commercially available (see, for example, reference (65)).

Most commonly, HPLC utilizes the ultraviolet absorbance detector (fixed 245 and 280 nm or continuously variable wavelength), refractive index detector, fluorescence detector, or traveling wire flame ionization detector. The mercury source of the UV absorption detector has a maximum output at 254 nm, so sensitivity is greatest for compounds that absorb at or near this wavelength. Other wavelengths are obtained, at the cost of some sensitivity loss, by interposing a filter between the lamp and cell or using a continuously variable, spectrophotometer type detector. Refractive index detectors are either based on the Christiansen effect or are of the prism type. These are universal, relatively insensitive (10^{-6} -10^{-7} g) detectors that require close temperature control and cannot be used with solvent programming. Fluorescence detectors are highly sensitive ($<10^{-9}$ g) and selective because of the choice of excitation and emission wavelengths. Derivatization (pre- or postcolumn) of the pesticide of interest is often required. The traveling wire flame ionization detector gives promise of universality and sensitivity like its GC counterpart, but it is at present mechanically complex and cumbersome.

6P THEORY AND PRINCIPLES OF HPLC

The principles and theory governing GC and LC are very similar, but the presence of a moving liquid instead of a gas gives far different separation characteristics to LC. The choice of the carrier gas in GC is primarily dictated by the type of detector used and has little influence on the separation achieved in a given column. In LC, the composition of the mobile phase is of prime importance in the thermodynamic distribution process. The other significant differences between GC and LC are that, in the latter, solute diffusion in the mobile phase is extremely low and temperature effects are of only secondary importance. Low diffusion in the mobile phase (a factor of 10^4 less than in GC) is the key reason why HPLC is possible to perform. Instead of plate height becoming increasingly larger as the carrier liquid flow increases as in GC, it becomes asymptotic to a limiting value. In practical terms, this means that the mobile liquid phase velocity can be increased without the same great loss in efficiency (increase in plate height) and loss of resolution that occur in GC. Temperature changes can differentially alter the relative retention of two similarly retained solutes by the effect on solubility, mobile phase viscosity, mass transfer effects, etc., but these are only indirect effects, and most LC separations are carried out at ambient temperature.

The concepts of retention time and resolution are the same in LC as in GC. Good resolution requires that peaks be narrow and the distance between peak maxima be great enough to allow the trace to return as nearly as possible to the baseline. Peak width is a function of the column efficiency (number of theoretical plates), while peak separation is a measure of column selectivity (the ability of the packing to differentiate between two solutes).

The basic equations for HPLC are the following:

$$\text{Retention:}\quad k' = \frac{V_1 - V_o}{V_o}$$

where V_1 is the retention volume for the peak of interest from the point of injection, and V_o is the retention volume of a non-retained peak measured at the peak apex. Times or distances measured along the recorder chart can be used more conveniently than volumes if the flow rate is constant.

Selectivity for compounds 1 and 2: $\alpha = \frac{k'_2}{k'_1} = \frac{V_2 - V_o}{V_1 - V_o}$

No. of Plates: $N = 16 \left(\frac{V_1}{W_1} \right)^2$

where W_1 is the width of the peak for component 1 (see Figure 4-C) in terms of V.

Resolution: $$R = \frac{1}{4} \underbrace{\left(\frac{\alpha - 1}{\alpha}\right)}_{\text{selectivity}} \underbrace{\left(\sqrt{N}\right)}_{\text{efficiency}} \underbrace{\left(\frac{k'}{1 + k'}\right)}_{\text{capacity}}$$

The k' term or capacity factor measures retention in column volumes; it is affected by the strength (e.g. polarity) of the solvent and strength (e.g. retentivity) of the column packing. The optimum value of k' is ca. 2-6. α is the separation or selectivity term, which is affected by the chemistry of the entire system, including the functionality of the sample components. Values of 1.1-2 are more typical in HPLC.

N is a measure of band broadening; typically, a highly efficient small-particle, porous 25-cm silica gel column will show approx. 10,000 plates for various compounds. The resolution equation combines terms associated with selectivity, efficiency, and capacity.

If the resolution of two components with $k' \cong 2$ or less is unsatisfactory, there are three different ways to try to improve the separation. The solvent can be changed (solvent strength lowered) to give k' values between 2 and 6, the column can be changed to increase N and give narrower peak widths, or the solvent can be changed to give increased selectivity (α). As a specific example, an inferior reversed phase separation on a 37-75 μ bonded phase C_{18} column with methanol/water solvent might be improved by changing to a lower percentage methanol (increase k'), changing to a 10 μ column (increase N), or changing to acetonitrile/water solvent (increase α).

In general, the best order for developing a separation is the following: try to dissolve the compound(s) of interest in a series of solvents ranging from hexane to water. If the only solubility is in the hexane end of the series, choose a silica gel column; if the only solubility is in the water end, use a C_{18} bonded column. If there is intermediate solubility, one has a choice of either column type. If the compounds to be separated are relatively polar and have functional group differences, silica gel is recommended. If the compounds are relatively non-polar and differ mainly in the hydrocarbon skeleton, a C_{18} column is recommended. Use the best available column to increase N, choose a solvent mixture and vary its proportions to alter k', and, finally, change the solvent composition while keeping the same strength to increase α.

Doubling column length doubles the number of theoretical plates, but the separation time will also double if flow rate is kept constant. Increasing pressure with a constant column length will increase the speed of separation but reduce resolution. A simple means of increasing the plate number is to reduce the solvent flow rate with a constant column length, but again we pay for this by increased separation time. Many operators seek maximum resolution by using the longest column and highest flow rate feasible at the pressure limit of the available instrument. Decreasing the eluting strength (e.g., polarity) of the solvent will usually increase resolution but will also increase the analysis time. Column efficiency is only marginally affected by column diameter: there is a small increase with increasing diameter, but diameter is principally important to sample loading capacity (sample size is proportional to the grams of active stationary phase available in the column). Doubling column diameter will approximately increase capacity by four, but four times as much solvent must flow through the column in a given time to maintain efficiency and velocity. A guide to selecting the best experimental conditions for high resolution in HPLC with large- and small-particle columns has been published (66).

6Q COLUMNS AND SOLVENTS FOR HPLC

Column efficiency is increased by using columns that are densely packed with uniform, small particles. Particles with an average size down to 30-40 μm can be successfully dry packed in the laboratory while microparticulates (5-10 μm) must be slurry packed. Because of the difficulty of this operation, commercial, pre-packed columns are usually used.

Pellicular packings have a thin layer or shell of stationary phase bonded to a solid glass core. The active layer can be silica, alumina, an ion exchange resin, or silica gel to which a "liquid" phase has been bonded (bonded-phase partition packings). The thin layer of stationary phase provides good mass transfer (efficiency) with a particle diameter that allows dry packing and low inlet pressure operation. A disadvantage of the thin active surface is reduced sample capacity. Totally porous microparticulates have very high efficiency because of their small average diameter (5-10 μ) and also have higher capacity than pellicular packings. They require high inlet pressure for acceptable flow rates and must be slurry packed. Present use of pellicular packings is mostly for situations where highest resolution and speed are not required, cost is a factor, or dry packing is desirable (microparticulates are at least one order of magnitude more efficient than pellicular packings). An exception to this is pellicular ion exchangers, which are often preferred to the microparticulate type.

For liquid-solid adsorption chromatography, microparticulate silica gel of irregular or spherical shape is used most. Alumina or other packings are used occasionally. Bonded phase packings are used for normal and reversed phase liquid-liquid partition separations. Reversed-phase chromatography on a monomeric or polymeric phase consisting of C_8 or C_{18} linear hydrocarbon covalently bonded to silica gel particles is by far the most widely used LC mode. Aqueous solvent mixtures are used as the mobile phase. This method owes its popularity to its ability to separate nonionic, ionic, and ionizable substances in partition, ion suppression, or ion pairing (67) modes; the stability of the bonded-phase columns (if properly used); and the simple, inexpensive solvent systems, utilized such as methanol or acetonitrile/water mixtures. Other reversed-phase bonded packings have phenyl and cyclohexyl groups, while normal phase bonded packings contain polar functionalities (e.g., nitrile, amine). A detailed discussion of HPLC columns and column technology has been published (68).

Solvents chosen for adsorption HPLC should have a low viscosity (for high efficiency), low boiling point (to facilitate sample recovery), adequate purity, low toxicity and odor, reasonable cost, and detector compatibility (low UV cutoff for the popular UV absorption detector). A widely versatile set of solvents with a range of chromatographic properties is hexane, methylene chloride, diethyl ether, acetonitrile, and methanol (most polar -- strongest eluent for normal phase adsorption chromatography). These solvents are usually used in mixtures with a solvent composition and strength that optimize capacity and selectivity (Section 6P). Solvent mixtures for adsorption HPLC of polar samples often contain at least a small concentration (0.01-1 percent) of a polar modifier (e.g., water, alcohol, acetonitrile) (69).

Many of the same considerations apply to the selection of solvents for the other important HPLC mode, reversed-phase chromatography on chemically bonded packings. Because the phases are reversed, water, the most polar solvent, is the weakest eluent, while neat methanol and acetonitrile are the strongest eluents used in most applications. The most polar solutes are the least retained on the column. Eluents of intermediate polarity are usually obtained by mixing methanol or acetonitrile with water or an aqueous acid, base, or buffer solution (70). One instrument manufacturer strongly recommends ternary solvent mixtures of varying composition, e.g., methanol, acetonitrile, and water (71). In general, silica gel reversed phase packings are stable only with solvents in the pH range of 2-7.5. In both adsorption and reversed-phase partition HPLC, gradient elution can optimize both resolution and speed for complex samples with components that cover a broad range of polarity (but resolution of any given compound pair is actually reduced compared to isocratic elution (72)).

Solvent selection for a particular separation is aided by two fundamental parameters, namely solvent strength (for control of k') and solvent chemistry (for control of α). Solvents have been tabulated according to their strength (polarity index) and chemistry (solvent group) for easy comparison (73).

6R PRACTICAL ASPECTS OF SUCCESSFUL HPLC OPERATION

All new columns should be tested with a standard mixture at standard chromatographic conditions to compare with the manufacturer's guarantee or previously used columns, and as a reference point for monitoring column changes with use. New columns must be fully equilibrated with the solvent (this can require up to several hundred void volumes with an adsorption column), and the column connections must have zero dead volume if constant retention times and high efficiency are to be achieved. The test mixture should contain pure compounds, one of which is nonretained plus at least two others that have $k' < 10$ and are well resolved so that as the column slowly degrades they will not overlap. The test mixture can contain pesticides to be analyzed in real samples with the same solvent system that is to be employed, or it can be a mixture specified by the column manufacturer so the performance data supplied with the (pre-packed) column can be verified. The concentration levels should be comparable to those to be used in the actual analysis.

Parameters monitored include absolute (k') and relative (α) retention plate number (N), asymmetry (tailing), void volume, and pressure drop. Small differences in α and k' usually reflect normal differences in solvent composition, but large decreases in these parameters or an increase in asymmetry are indications of column degradation or deactivation. Both channeling and compression of the packing can also increase. If the void volume decreases, channeling may be occurring or the packing pores may contain gas bubbles or immiscible liquid. Changes in pressure drop indicate channeling, plugging, or leaking. Buildup of impurities from the solvent or samples will eventually cause loss of column efficiency, which can usually be restored by regenerating the column with a series of solvents of increasing eluting strength (adjacent solvents must be miscible). The solvent sequence is then reversed, and each solvent is followed by a weaker one. A possible solvent sequence for regenerating adsorption (normal phase) columns is methylene chloride-methanol-water-methanol-dry methylene chloride-dry hexane (20 column volumes each). Acetonitrile is an effective solvent for regenerating reversed phase bonded packings. The pump and connecting tubing are pre-washed with each new solvent that is put through the column, high flow rates are used to speed the operation, and the detector is left connected, if possible, to also clean it.

The inevitable, permanent column degradation that occurs with prolonged use can be retarded if proper precautions are taken for sample cleanup, solvent preparation, periodic column regeneration, and storage. The manufacturer's literature should always be carefully studied and recommendations faithfully followed. Prevention of plugging is probably the one most important precaution that must be exercised to prolong column permeability and efficiency. Removal of particles from solvents is discussed along with other aspects of solvent purity in Section 3I. Sample extracts or solutions should also be free of insoluble particles (74) and should be filtered, if necessary, with a hypodermic syringe fitted with a Swinny-type filter (0.5-1 μ). Irreversibly sorbed compounds can irrepairably damage the column and, if present, can be removed on a short (5-10 cm) guard column located between the injector and the analytical column. In order not to sacrifice separation efficiency, the guard column should be of the same diameter and packing as the main column. Less expensive, easily dry-packed guard columns can be prepared from ≃40 μ pellicular sorbents, but some efficiency may be lost if the analytical column contains microparticulates. Columns damaged by plugging, bad compression, or irreversibly sorbed material can sometimes be returned to original efficiency by removing the column inlet fitting and frit and replacing the discolored packing and deposited material with fresh packing. The same packing material should be added by the appropriate dry or slurry packing procedure, or, alternatively, a methanol slurry of the packing can be added dropwise and allowed to settle into place. The end frit is cleaned before replacement by immersion in an ultrasonic bath for a few minutes.

HPLC columns should never be bumped, dropped, jarred, bent, tapped, or vibrated. All connection fittings must be clean (use an ultrasonic bath). Fittings are never over-tightened or they will become distorted and eventually leak. Columns should be stored tightly capped in a compatible solvent. Silica-based bonded reversed phase packings are never stored in aqueous solution but are flushed and stored in methanol or acetonitrile. If the column has been used with a buffer, it is flushed with water and then the organic solvent. Aqueous solutions can be left flowing slowly overnight (5 ml/hour) for use the next day, but the column should not remain static in aqueous solution.

Some common problems in adsorption LC and possible means for their correction (69) follow. Baseline drift can be caused by strongly adsorbed peaks eluting from an earlier run. Such drift is remedied by pumping through the column at the end of each run several column volumes of strong solvent (isocratic elution) or solvent of higher final strength (gradient elution). Baseline drift can also be caused by incomplete system equilibration in switching from one solvent to another. This problem is most acute with the RI detector. Spurious peaks can be caused by bubbles in the detector or impurities in water or other solvents. Bubble formation is avoided by prior solvent degassing or installation of an in-line backpressure valve generating 50 psi to keep all gases in solution. Peak tailing is more common in adsorption HPLC and is often caused by insufficient adsorbent deactivation. Use of a modifier in the solvent can correct this problem. Partial ionization of the sample can cause tailing that can be suppressed by changing the solvent pH or ionic strength. Injection of the sample in a solvent stronger than the eluent can also cause tailing; a solvent weaker than the eluent, or more preferably the eluent itself, should always be used, if possible, or the sample may be injected in a very low volume of a stronger solvent. Drifting retention times can be caused by differences among solvent batches, changes in composition of a batch of solvent on standing, changes in flow rate or temperature, or inconsistent adsorbent activity. Adsorbent activity is maintained constant by using clean samples, pure solvents with an adequate level of modifier, and frequent column regeneration. Poor reproducibility of retention times and peak areas in gradient elution may arise from inadequate column regeneration between runs, nonreproducible pump delivery, or poor mixing in the mixing chamber.

Dirty detector flow cells may be cleaned by using a 10 ml syringe to rinse the cell successively with methylene chloride, methanol, and water. The cell is then filled with 50 percent nitric acid and allowed to stand for 30 minutes, followed by flushing with filtered, distilled water (75). If this operation does not reduce the background to an acceptable level, either other problems are involved (e.g., cell misalignment or an impure or inappropriate solvent) or the detector must be disassembled and cleaned further in accordance with the manufacturer's instructions.

Quantitation in HPLC with UV detection is best carried out using peak heights if solvent composition can be maintained precisely but flow control is poor. Peak areas are used when flow rate is stable but the composition of the mobile phase might vary (as would be common in adsorption chromatography where traces of water and polar contaminants are difficult to control) (76).

Readers using HPLC for analysis of pesticide residues are strongly urged to study the excellent discussion of many practical aspects of the field given in reference (69), from which much of the material in this subsection was taken.

6S APPLICATIONS OF HPLC TO PESTICIDE ANALYSIS

The material presented on HPLC has been expanded in the present revision of this manual because of the increasing importance of the technique in residue analysis. Even greater coverage is anticipated in future revisions as HPLC becomes more sensitive, practical, and reliable in the multiresidue analysis of field samples. For further information on HPLC, readers are referred to reviews describing LC detectors (77-79), column packings (68), and general principles and equipment (80, 81), and to books covering theory, principles, and practice (82, 83). A scheme has been published for isolating and troubleshooting instrument and column problems in HPLC utilizing a glass-bead column and two simple electronic checking devices (84).

A novel method with great promise for the simplified monitoring of residues in water samples down to ppt levels has been termed "trace enrichment". The procedure combines concentration, separation, detection, and quantitation of nonpolar to moderately polar impurities. The water to be analyzed is pumped through a C_{18} reversed phase column until a sufficient quantity of impurities has been deposited at the top of the column. (In a reversed phase system, water is the weakest possible eluent, so the organic compounds will be concentrated in a tight band at the head of the column). A gradient elution from 100 percent water to 100 percent methanol or acetonitrile is then performed, during which the organic impurities are eluted sequentially in order of their polarity (most polar is first eluted) and detected with a UV absorption detector (85).

Table 6-1 contains some other recent applications of HPLC to pesticide analysis, selected to illustrate the range of pesticide types and samples that have been studied. Most analyses to date have been developed for one or a few specific residues in food or crop samples, and many analyses of formulations and technical material now involve HPLC as the determinative step. For example, 1977 issues of the Journal of the AOAC have reported HPLC formulation determinations of Folpet, esters and salts of 2,4-D, methyl parathion, and methyl pirimiphos. Reviews of earlier applications of HPLC to pesticide analysis are cited in References (63, 108-111).

Attempts have been made to combine HPLC and mass spectrometry for the direct analysis of column effluents. Approaches to this direct coupling have included (a) use of atmospheric pressure ionization; (b) enrichment of reversed-phase effluents using a dimethyl silicone membrane interface; (c) chemical ionization using a small fraction of the carrier solvent as reagent gas; (d) transport of solute through differential vacuum locks on a wire or a metal or plastic ribbon; (e) reduction of solute to hydrocarbon and subsequent FID and MS analysis; and (f) formation of a molecular beam by laser vaporization of solvent. At present, (c) and (d) are the most popular methods, but none is free from major disadvantages and none has yet been tested for routine pesticide analysis. The various methods and some applications of HPLC-MS have been reviewed in detail (112). A simple off-line coupling procedure has also been described (113).

Table 6-1

SELECTED SEPARATIONS AND DETERMINATIONS OF PESTICIDES BY HPLC

Compound determined	Sample matrix	Column type	Solvent system	Detector	Cleanup	Recovery	Residue level	Reference
TH-6040 insect growth regulator	bovine manure	C_{18} reversed phase	acetonitrile-water (57:43)	UV, 254 nm	Florisil column	97%	0.5 and 2 ppm	(86)
Carbamate insecticides and metabolites	vegetables	C_{18} reversed phase		fluorescence, after dynamic hydrolysis and labeling	partition	69-90%	0.01-1 ppm	(87)
Carbofuran and 3-OH and 3-keto metabolites	crops	5μm silica gel, adsorption	3-8% isopropanol in isooctane	UV, 254 or 280 nm	partition and Florisil column	68-110%	0.1 and 1 ppm	(88)
17 Carbamate and urea pesticides	standards only	Partasil-10 ODS reversed phase	different polarity solvents	UV, 254 nm	--	--	--	(89)
5 fungicides (benomyl, phenylphenol, biphenyl, thiabendazole, carbendazim)	fruits	adsorption, reversed phase, ion exchange	various, compatible with column	UV, 254 or 288 nm	steam distillation, partition, chemical reaction	64-102%	0.8-100 ppm	(90)
Difenacoum and Warfarin	liver, plasma, urine	Corasil II pellicular, adsorption	isopropanol-$CHCl_3$-isooctane (1:2:397)	UV	GPC or partition	62-90%	0.025-5 ppm	(91)
Methyl parathion	runoff water	Partasil ODS, reversed phase	acetonitrile-water (50:50)	UV, 270 nm	XAD-2 for preconcentration	99%	2-3 ppm	(92)

Table 6-1 (continued)

Warfarin	biological fluids	Micropak CH-10	methanol-0.5% acetic acid (1:1)	UV, 308 nm	freezing of aqueous phase	(2-5% C.V.)	0.1-4 µg/ml	(93)
Azinphos methyl oxon	foliage soil	Corasil-uC$_{18}$, reversed phase	acetonitrile-water gradient	UV or on line colorimetric reactor	none	96%	1-10 ppm (soils)	(94)
Phenylurea herbicides	soil, river water	Spherisorb ODS, reversed phase	methanol-water-NH$_3$	UV	none	90-100%	0.5-1 ppm	(95)
Karbutilate and metabolites	water, soil, grass	µPorasil, adsorption	3-7% ethanol in ethylene dichloride	UV, 254 nm	Florisil column (for grass extracts)	80-103%	0.1-0.2 ppm	(96)
Triazine, urea, uracil herbicides	standards	PE C$_{18}$ Sil-X-11, reversed phase	2.5-25% methanol in water	UV, 200-300 nm	--	-	Sensitivity 0.05 ppm in soil and 0.001 ppm in water stated	(97)
Warfarin and metabolites	blood, plasma and liver microsomes	µC$_{18}$, reversed phase	1.5% acetic acid-acetonitrile (69:31)	UV, 313 nm	on-column concentration	≃100%	0.04-0.08 µg/µl	(98)
Carbamate pesticides (Swep, Landrin, carbofuran, aminocarb, Banol, carbaryl, Zectran, methiocarb, propoxur, Mobam)	foods	5 µm silica gel, adsorption	5% isopropanol in isooctane	UV, 254 nm	partition and Florisil column	>70%	0.1-0.3 ppm	(99)
PCBs	commercial mixtures	5 µm silica gel adsorption	dry hexane	UV				

Table 6-1 (continued)

Urea herbicides (linuron, chlorbromuron, chlroxuron, fenuron, etc.)	vegetables and wheat	5 µm silica gel, adsorption	5-20% isopropanol in isooctane	UV, 254 nm	partition and Florisil column	>80%	0.01-1 ppm	(101)
Benomyl and TBZ	plant tissues	20-23 µm silica gel, adsorption	0.1% acetic acid in methanol	fluorescence	benomyl hydrolyzed to 2-AB	90-103%	ca. 0.01-.2 ppm	(102)
Paraquat or diquat	urine	α-amino-propyltri-ethoxysilane bonded to 20 µm alumina	pH 2.45 phosphate buffer-methanol (11:14)	UV, 258 nm	none	ca. 100%	650 µg/L	(103)
Dioxins	PCP	Permaphase-ODS, reversed phase	14% H_2O in methanol	UV	extraction, ion exchange column	93-104%	0.7-41 ppm	(104)
Terbutryne	water	Permaphase-ETH, aliphatic ether, chemically bonded pellicular	20% methanol in water	UV	none	89-100%	0.001-0.1 ppm	(105)
Chlortoluron	soil	10 µm silica gel, adsorption	15% isopropanol in hexane	UV, 240 nm	silica gel column	71-95%	0.25-2.5 ppm	(106)
Methazole, DCPMU, and DCPU	plant and animal samples	Zorbax SIL, adsorption	0.05% methanol in dichloromethane or $CHCl_3$-petroleum ether-methanol (6:3:0.5)	UV, 254 nm	partition and silica gel column	75-105%	0.1-0.2 ppm	(107)

THIN LAYER CHROMATOGRAPHY

6T INTRODUCTION

The first multiresidue method available to the pesticide analyst for identification and estimation was based on paper chromatography (114-116). Paper chromatography has now been largely replaced by thin layer chromatography (TLC) since the latter will generally give faster and more efficient separations with better spot definition and greater sensitivity. The use of paper chromatography in pesticide residue analysis has been reviewed (116-119).

TLC is used mainly for confirmation of residues following initial screening and quantitation by GC. Confirmation by TLC, which is based on comparison of migration distances of the pesticide of interest with authentic standards run on the same layer, is covered in Subsection 8E in Section 8 of this Manual. In addition, TLC may be used as a screening procedure followed by confirmation and quantitation using GC, or the quantitation can be carried out by TLC if a gas chromatograph is not available or if the pesticide of interest is unstable during GC. Extraction, cleanup, and concentration steps normally precede TLC determination. Often more stringent cleanup is required for TLC than for GC if streaked zones are to be avoided. For example, the 15 percent diethyl ether fraction from the Florisil column cleanup of a fat sample contains a large amount of lipids. Although adequate for GC, further TLC or column cleanup prior to TLC is required (EPA PAM, Section 12,B,V). TLC has also been occasionally used for cleanup of extracts prior to determination by GC (120).

Major advantages of TLC are simplicity, rapidity, and low cost. Sensitivity ranges from about 5-500 ng for most pesticide detection methods. Rapid semi-quantitative estimation can be achieved by visual comparison of sample and standard spot sizes and/or intensities, and more accurate quantitation can be carried out by *in situ* scanning of spots with a spectrodensitometer.

This section will briefly survey important aspects of TLC for screening and quantitation of pesticide residues. General techniques of TLC were described in detail in an extensive treatise (121), while specific procedures for pesticide TLC were covered in several papers (117, 122). Applications of TLC to pesticide analysis have been reviewed (123, 124).

6U PRACTICAL CONSIDERATIONS IN TLC

Spots are applied to the thin layer using simple disposable capillaries, GC syringes, or automatic multiple spotting devices. All initial zones should be of small, uniform size and only enough pesticide spotted to allow for detection after the run. Care should be taken that the spotting pipette does not penetrate the surface layer. Standard solutions must be spotted on the same plate as the sample, preferably on both sides of the sample spot.

Layers are hand-coated with a commercial adjustable spreading device (Figure 6-I) or purchased pre-coated on glass, plastic, or aluminum backing. Analytical layers are usually 250 μm thick. Pre-coated layers are of high purity and uniformity and are used almost exclusively in most laboratories, especially for *in situ* quantitation by densitometry. Substitution of one brand of adsorbent for another or pre-coated for hand-coated plates often cannot be directly made. For example, silica gels with differing polarities or surface hardness (binders) may require modified solvent systems or detection reagents if similar results are to be obtained. Activation of adsorbent layers (e.g., 80-110°C for 30-60 minutes) prior to spotting and development is often required. Once the adsorbent has been activated, it must either be used promptly or stored under desiccating conditions, or activation must be repeated. Silica gel and alumina layers usually give the best results, but polyamide, microcrystalline cellulose, kieselguhr, zinc carbonate (125) and magnesium oxide among other adsorbents have also been used. For reversed phase TLC, hydrophobic C_{18} chemically bonded silica gel plates are commercially available.

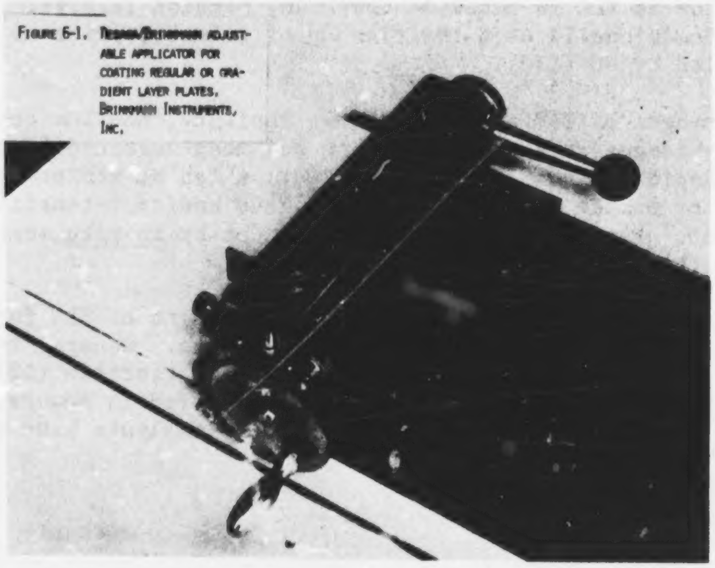
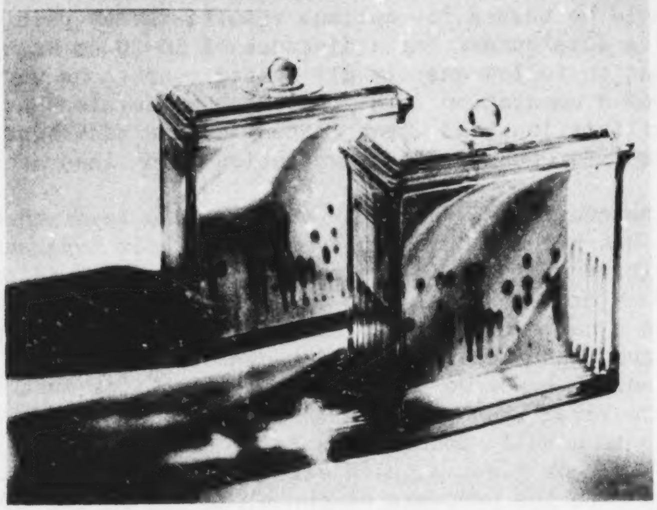


Figure 6-1. Desaga/Brinkmann adjustable applicator for coating regular or gradient layer plates. Brinkmann Instruments, Inc.

Chromatography is carried out in a development chamber, most often a rectangular, glass, paper-lined tank saturated with solvent vapors (Figure 6-J). Low volume "sandwich" chambers are also used. Both saturated and unsaturated atmospheres have been used to advantage and should be tested for optimum results in any particular application. Ascending development for a distance of 10-20 cm is typical. It is important to follow exactly all stated conditions when attempting to reproduce a separation. The temperature, development chamber design and equilibration, and water content of the adsorbent are probably the most frequent sources of variation among laboratories.

The technique termed "high performance thin layer chromatography" (HPTLC) has become increasingly important for separations and in situ quantitative analysis in the recent past. HPTLC is carried out on 10 x 10 cm or 5 x 5 cm layers of silica gel with a smaller particle size and a narrower particle size distribution than in conventional TLC plates, and thereby gives improved resolution and sensitivity of detection. Volumes no larger than 1 microliter must be spotted for these advantages to be fully realized. For manual application, spotting is usually done with a Pt-Ir tipped Nanopipet (or equivalent), or this type of pipet is used with an automatic spotting device that controls both the pressure of the pipet tip on the layer and the duration of contact. Solvent development is carried out in a miniature glass rectangular chamber or in a commercial, automatic U-chamber device producing radial zones (126) (a special radial scanner is needed to quantitate these separations). High resolution is achieved rapidly with short development distances. In a typical residue analysis, it is virtually impossible to apply the whole cleaned-up sample extract or an appropriate, accurate aliquot as a spot of 1 µl or less, so HPTLC has not yet been widely used for actual samples. New approaches are appearing that may solve this problem by allowing a larger sample to be applied without sacrificing the benefits of the HP layer. One proposed solution utilizes a two-section plate with a high performance analytical layer above a spotting region; initial development concentrates the diffusely applied sample into a narrow zone at the interface of these layers (127). Another possibility is the use of programmed multiple development (apparatus from Regis Chemical Co.), which causes large initial spots to be narrowed during migration on the HP layer (128). HPTLC plates are available from Merck and Quantum Industries, and HPTLC equipment from Camag, Inc.

Figure 6-J. Desaga rectangular TLC tanks.
Brinkmann Instruments, Inc.



The following solvent systems have proved to be generally useful for separation of a wide range of pesticides on silica gel thin layers: benzene mixed with varying amounts of ethanol for polar compounds or with hexane for those which are less polar; and a mixture of hydrocarbon plus acetone plus chloroform, with the addition of methanol for more polar pesticides. Examples include pentane-acetone-chloroform (65:30:5 v/v) or pentane-acetone-methanol-chloroform (70:15:10:5 v/v). The purpose of the chloroform is to control the evaporation of acetone in the atmosphere of an unsaturated tank. Proportions of the components are changed to suit the requirements of specific separations.

After development and air drying of the layer, spot detection may be achieved in a number of ways. Few pesticides are naturally colored, but colored derivatives may be made prior to spotting, e.g., dyes formed from aromatic amine moieties of urea herbicides by coupling with N-ethyl-1-naphthylamine [FDA PAM, Vol. II, Sec. 120.216]. Colorless spots can be detected by applying a chromogenic reagent, either by spraying or dipping. A commercial aerosol spray device is shown in Figure 6-K. Dipping is the preferred method of application, if feasible, because of the uniformity achieved and the hazards involved in careless spraying of corrosive, toxic, or carcinogenic reagents. A Thomas-Mitchell dipping tank is recommended. Sometimes the reagent can be incorporated in the layer prior to development or included in the developing solvent. Naturally fluorescent spots can be detected under short (254 nm) or long (366 nm) wave UV light, or fluorescence may be induced by application of fluorogenic reagents after development or preparation of fluorescent derivatives (e.g., dansyl compounds) prior to spotting (129). Spots that absorb UV light are detected as quenched (dark) spots on layers containing phosphor activated by UV light (usually 254 nm). Radioactive (labeled) pesticides are detected by autoradiography and some fungicides by direct bioautography.

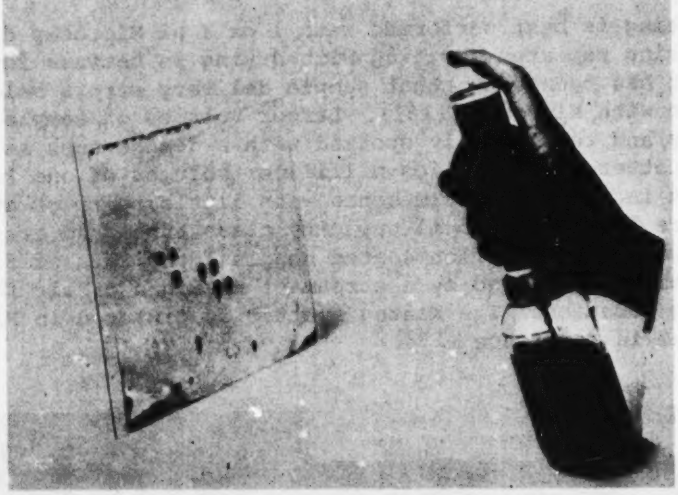


FIGURE 6-K. TLC AEROSOL SPRAYER. BRINKMANN INSTRUMENTS, INC.

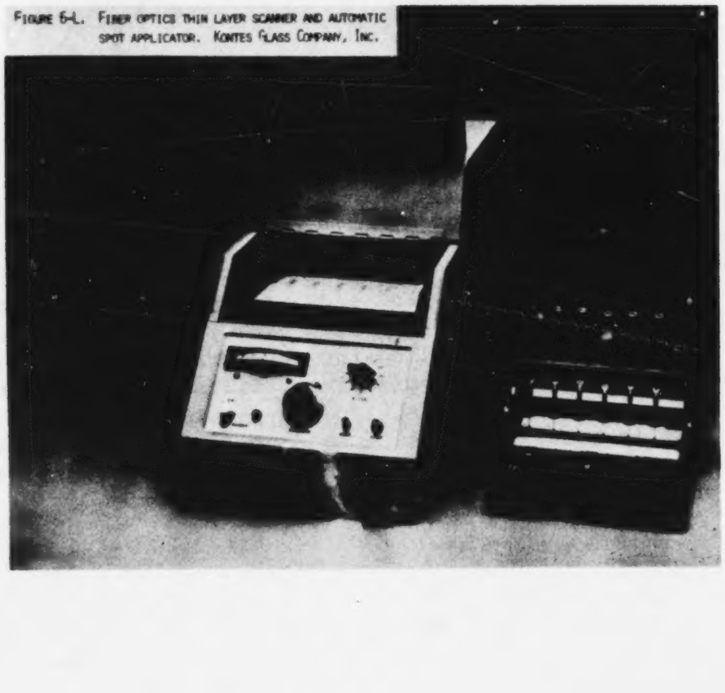
6V QUANTITATIVE TLC

Quantitation of separated spots may be achieved by "eyeball" comparison between sample and standard spots run on the same plate or by some independent analytical method (e.g., spectrophotometry or GC) after scraping the spot, collecting, and eluting the pesticide from the adsorbent. Manual elution is simply carried out by scraping the area containing the pesticide spot, collecting the scrapings in a vial or tube, adding solvent and agitating (vortexing), filtering the adsorbent, and concentrating the filtrate containing the pesticide. An automated elution system is available from Camag, Inc.(130). Radioactive spots can be quantitated by scintillation counting after scraping or by automatic scanning of radioactivity on the layer.

Colored, fluorescent, or quenched spots may be scanned on the layer when a spectrodensitometer is available. Quantitation is achieved by scanning sample and standard spots in the optimum instrumental mode and treating the resultant peaks, representing the amount of light absorbed or emitted, in the same manner as GC peaks for calculations. A versatile densitometer is capable of scanning in single or double beam and reflectance or transmission modes, and has monochrometers or filters for selection of the best wavelengths of incident and emitted (for fluorescence) energy. Important considerations for densitometry are precise and accurate spotting, uniform layers, R_F values between 0.3 and 0.7, uniform application of detection reagents, and optimum use of a good densitometer.

Manual spotting is best performed with 1 or 2 µl Microcap disposable pipettes, using repeated spotting with drying in between for larger volumes. It has been shown that sample delivery errors below 1 percent are feasible with Microcaps (131). Larger volumes of sample extracts are conveniently and reproducibly spotted with a device such as the Kontes automatic spotter which applies milliliter volumes of one to six samples or standards in small, uniform zones with little operator attention. Solutions are loaded into 5 ml capacity glass tubes and are delivered onto the layer through Teflon coated needles, the rate of flow and spot size being controlled by a stream of nitrogen or air focused onto the spotting location. The Kontes spotter is pictured in Figure 6-L and described in reference (132).

FIGURE 6-L. FIBER OPTICS THIN LAYER SCANNER AND AUTOMATIC SPOT APPLICATOR. KONTES GLASS COMPANY, INC.



Pre-coated layers are recommended for quantitative TLC since it is very difficult to hand-coat layers with adequate uniformity. Uniform application of chromogenic or fluorogenic reagents is better achieved by dipping than by spraying. However, dipping is not always possible; its use depends on the reagent solvent, adsorbent, and type of compounds on the layer.

When developing a new densitometric method, the spots of interest should be scanned in all possible modes and directions and at a variety of wavelengths in order to obtain the best signal to noise ratios and selectivities. The optimum conditions are then used to obtain the calibration curve (linear range) and perform the analysis. Samples and bracketing standards should always be chromatographed on the same plate.

Thin layer densitometry is capable of precision of 1-2 percent on a routine basis and can rival GC for determination of certain pesticide residues in the hands of an experienced operator. Principles and experimental details of thin layer densitometry have been published (133), and quantitative TLC of pesticides has been reviewed (134). Table 6-2 contains some selected applications of thin layer densitometry reported since this review was published. A fiber optics scanner specifically designed for pesticide analysis (135) is available from the Kontes Glass Co. at a modest price (Figure 6-L).

6W THIN LAYER SYSTEMS

a. Chlorinated Pesticides

Extracts of fatty and nonfatty foods cleaned-up on a Florisil column are chromatographed on prewashed alumina layers developed with heptane (for the 6 percent diethyl ether-petroleum ether Florisil eluate) or 2 percent acetone in heptane (15 percent diethyl ether fraction). Detection is provided by spraying with $AgNO_3$-2-phenoxyethanol reagent in ethanol or acetone and exposing to high intensity short-wave UV light to produce brown to purplish-black spots. The construction of a UV light apparatus containing four 15 watt lamps for rapid color development and allowing a variable distance between the TLC plate and the light source is described in the Canadian PAM, Section 14.10. Thin layer media must be very low in chlorine content, and other precautions and care must be taken to prevent large areas of the plate from turning brown or grey and thereby reduce the contrast of the spots with background. A sensitivity in the 5-500 ng range is possible with $AgNO_3$ reagent, with a light steaming before spraying often aiding the detection. Conventional 8 inch by 8 inch glass plates, commercial pre-coated TLC sheets, or 3-1/4 inch by 4 inch microslides may be employed. Complete details of these methods plus R_F values for numerous

TABLE 6-2 PESTICIDES QUANTITATED BY THIN LAYER DENSITOMETRY

Compounds	Sample matrix	Scanning mode	Detection method	Reference
Captan, captafol	apple, potato,	fluorescence	$NaClO_3$	(136)
Benomyl, carbendazim, and 2-AB	fruits, vegetables	quench	fluorescent layers	(137)
Herbicides containing NH_2 or OH groups	water, soil	fluorescence	dansyl chloride	(138)
s-Triazines	standards only	quench	fluorescent layers	(139)
Fenitrothion	water	fluorescence	$SnCl_2$/fluorescamine	(140)
Chlorophenoxy acid herbicides	water	visible	$AgNO_3$	(141)
OCl pesticides	human autopsy samples	visible	$AgNO_3$	(142)
OP pesticides	tissues	visible	palladium chloride	(143)
Acidic herbicides	standards only	fluorescence	4-bromoethyl-7-methoxycoumarin	(144)
OP insecticides	standards only	visible	$AgNO_3$ or enzyme inhibition	(145)
Coumaphos	water	fluorescence	heating	(146)
OP pesticides	water	fluorescence	hydrolysis/dansyl chloride	(147)
Maretin	milk, eggs	fluorescence	--	(148)
Quinomethionate	crops	fluorescence	--	(149)
Coumaphos and O-analog	eggs	fluorescence	heating	(150)
Thiabendazole	fruits	fluorescence	--	(151)
Chloramben	bean, tomato	visible	Bratton-Marshall reagent	(152)
Bayrusil	foods	fluorescence	heating	(153)
DDT	water	visible	$AgNO_3$	(154)
Carbaryl	potato	visible	p-nitrobenzenediazoniumfluoborate	(154)

compounds in the aforementioned two solvent systems, as well as for an alternate system, consisting of immobile dimethylformamide on alumina and isooctane as the developing solvent, are given in Sections 410, 411, and 413 of the FDA PAM. Silver nitrate has been incorporated into acid-washed alumina before coating the plates so that only exposure to UV light is required for spot visualization (FDA PAM, Section 412). The $AgNO_3$ detection method has recently been studied in detail for the determination of chlorinated insecticides and herbicides (155).

Similar TLC procedures are described in detail in the EPA PAM, Section 12,B for the determination of chlorinated pesticide residues in serum and adipose tissue. An extract from 50 grams of serum, cleaned-up on Florisil and concentrated to 100 µl before spotting, will produce a visible spot at 2 ppb, assuming that 10 ng of pesticide is detectable. An adipose tissue extract from a 5 gram sample, concentrated to 500 µl, will give a readable spot at 10 ppb. The method involves TLC of the 6 percent and 15 percent Florisil column eluates as above, with additional prior cleanup of the 15 percent fraction on an alumina microplate developed with acetonitrile.

Silica gel layers developed with hexane, 1 percent acetone-hexane, 10-50 percent benzene-hexane, or 1 percent ethanol-hexane are recommended for screening chlorinated pesticides in foods at 0.1 ppm levels [Canadian PAM, Procedures 0.1 and 12.4]. Complete details of plate preparation, extract concentration, and visualization with silver nitrate reagent are given in this source, along with figures of spot locations for eleven common pesticides in four mobile solvents.

For complex pesticide mixtures, two dimensional or multiple development techniques may be helpful. The former was used to identify organochlorine pesticides in blood and tissues (156) and the latter (157) for the separation of 13 common pesticides.

Extensive listings of additional solvent systems and corresponding R_F values for chlorinated pesticides will be found in references (123, 158, and 159). The thin layer densitometry of chlorinated pesticides after spraying with silver nitrate reagent is described in Chapter 15 of reference (133).

b. Organophosphorus (OP) Pesticides

Cleaned-up extracts may be developed with methylcyclohexane on DMF-coated alumina layers and detection made by spraying with tetrabromophenolphthalein ethyl ester, $AgNO_3$, and citric acid. This reagent reacts only with thiophosphoryl compounds to give blue or magenta spots [FDA PAM, Section 431; EPA PAM, Section 12,B]. Thio and nonthio organophosphates are developed on silica gel layers with isooctane-acetone-chloroform (70:25:5 v/v) and detected as blue or magenta spots by treatment with p-nitrobenzyl pyridine and tetraethylpentamine spray (FDA PAM, Section 432].

A two dimensional procedure (160) has the significant advantage of specificity, obtained by oxidation of the OP pesticides with bromine before development in the second direction. Silica gel layers with toluene, 25 percent heptane in ethyl acetate, or ethyl acetate as developing solvents were used along with the Storherr charcoal column cleanup procedure (Subsection 7Q in Section 7) and enzymatic detection to identify 18 pesticides in crops at 0.01 ppm levels. The same procedure should be well suited to OP pesticides in human and environmental samples after appropriate cleanup.

Enzyme inhibition techniques are important for the selective and sensitive (pg-ng amounts) detection of enzyme inhibitors such as OP and carbamate insecticides and metabolites. These compounds inhibit esterases and thereby prevent hydrolysis of a chromogenic substrate. Procedures include separation by TLC (on silica gel layers sometimes as thick as 450 μm), optional treatment with bromine vapor or UV light, and spraying of the layer with enzyme and substrate solutions. Areas corresponding to inhibitors are visible as white spots on an intensely colored background; i.e., inhibited enzyme is surrounded by enzyme free to hydrolyze the substrate and thus produce color. While many OP pesticides are inhibitors *per se*, bromine or UV treatment is required to convert others to active inhibitors. For carbamates, UV or bromine treatment may produce no change or increased or decreased inhibition, depending on the compound. Sample extracts often require minimal cleanup prior to TLC analysis with enzymatic detection; for example, hexane extracts of many foods can be directly chromatographed. Section 9.2 of the Canadian PAM provides procedural details, tables of sensitivities and effects of bromine and UV treatment for OP and carbamate pesticides, and diagrams of mobilities with hexane-acetone (8:2 v/v), a generally useful development solvent for TLC on 450 μm silica gel layers. The preparation of these layers is detailed in Section 12.4 of the Canadian PAM. Several different esterases have been compared for the detection of 65 OP and carbamate pesticides in vegetables and fruits (161).

TLC enzyme inhibition methods and applications to pesticides have been reviewed (162-164) as have the merits of TLC for analysis of residues (165). The separation and detection of 42 phosphate compounds using five ternary solvent systems on three adsorbents and three selective chromogenic sprays have been reported (166). Twenty five solvent systems and several visualization reagents were evaluated for detection of 12 OP insecticides in tissues (167).

c. CHLOROPHENOXY ACID HERBICIDES

Extracts containing methylated chlorophenoxy acids are cleaned-up on a Florisil column and chromatographed on alumina layers using hexane saturated with acetonitrile as the developing solvent. Cleaned-up extracts containing free acids are developed for a distance of 3.5 cm on a pre-coated silica gel sheet with cyclohexane-acetic acid (10:1 v/v), then the sheet is dried and developed for 15 cm in the same direction with benzene-petroleum ether (3:1 v/v). Spraying with silver nitrate chromogenic reagent produces black spots with a sensitivity of ca. 50 ng for the esters and 100-500 ng for the free acids. Details of both methods and R_F values are given in Sections 421 and 422 of the FDA PAM. Other detection reagents for these pesticides include Rhodamine B and Bromocresol green indicators (168).

d. Other Pesticide Classes

The TLC of other classes of pesticides including carbamates, ureas, phenols, dithiocarbamates, triazines, and organomercurials was reviewed in references (123) and (124). Applications, solvent systems, detection, and quantitation are covered in these references. TLC is particularly applicable to herbicides, many of which are polar and not susceptible to gas chromatographic analysis without derivative formation. Studies have been reported for the TLC of triazine herbicides on silica gel (139, 169) and polyamide (170); determination of 11 urea herbicides in water (171); detection of dithiocarbamate fungicides with congo red (172); and separation of carbamate and phenylurea pesticides on polyamide (173).

The TLC of five dithiocarbamate residues in chloroform extracts of leaves is detailed in Section 9.3 of the Canadian PAM. Silica gel layers developed with benzene for dimethyldithiocarbamates or acetic acid-methanol-benzene (1:2:12 v/v) for ethylenebisdithiocarbamates are used, with detection as yellow, brown, or green spots after a cupric chloride-hydroxylamine hydrochloride spray.

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Section 7

MULTIRESIDUE EXTRACTION AND ISOLATION PROCEDURES FOR PESTICIDES AND METABOLITES

This section will present brief descriptions and problem areas of widely used multiresidue analytical procedures for different sample substrates. A few methods for important individual residues will also be included. Many of these problems are treated elsewhere in this Manual but are highlighted again here to alert the analyst using the different methods. References will be given in each case to sources of detailed methodology. Control of procedures for collection of samples for these methods is covered in Subsections 6A - 6K in Section 6, while general comments on sample extraction and extract concentration will be found in Subsections 6L and 6M in Section 6.

CHLORINATED PESTICIDES

7A TISSUE AND FAT ANALYSIS BY A MODIFIED MILLS, ONLEY, GAITHER PROCEDURE

The macro method described in Section 5,A,(1) of the EPA Pesticide Analytical Manual has been determined by a number of interlaboratory collaborative studies to yield very acceptable precision and accuracy for the analysis of a number of chlorinated pesticides and metabolites in human or animal fatty tissues. However, many polar OP and carbamate pesticides are not recovered. This method involves grinding of a 5 gram sample with sand and anhydrous Na_2SO_4, isolation of fat by repeated extraction with petroleum ether, re-extraction of residues into acetonitrile and then partitioning back into PE after adding 2 percent NaCl, drying by elution through a column of Na_2SO_4, concentration of the eluate, cleanup on a Florisil column, and EC-GC after reconcentration of column eluates. If necessary, further cleanup of the 15 percent ether-petroleum ether Florisil eluate is carried out on a MgO-Celite column. Pooled blood serum can be analyzed by the MOG Florisil procedure after extraction with a hexane-acetonitrile solvent system [EPA PAM, Section 5,A,(3),(a), VIII].

In addition to being complex, this method is highly empirical in that failure to follow the methodology as described may lead the analyst into problems of residue identification and quantitation. Some of the method pitfalls observed during the operation of the interlaboratory quality control program (Section 2) are as follows:

a. Some analysts, with the mistaken notion of saving time, have combined 6 percent and 15 percent ethyl ether-petroleum ether Florisil column fractions, and have then attempted gas chromatography on the mixture. With some luck this might prove successful, but there is a high probability of being unlucky. For example, in one documented instance, an analyst reported the presence of aldrin in a human fat sample. Other collaborators on the sample analysis found the same peak but in the 15 percent eluate, negating the possibility of aldrin since this compound elutes wholly in the 6 percent fraction. By combining the fraction extracts, the analyst inadvertently neglected one valuable identification tool, that of selective adsorption.

b. The polarity of the ethyl ether-petroleum ether eluting solutions exerts a profound effect on the elution pattern of several pesticidal compounds. The amount of ethanol, a relatively polar solvent, in the ethyl ether is a critical factor as illustrated in Figure 4-A in Section 4. As indicated in this figure, with no ethanol, dieldrin would be expected to yield only 87 percent recovery in Fraction II with the balance being retained on the column. If twice the proper amount of ethanol is present, approximately 7 percent should elute in Fraction I, giving a 93 percent recovery in Fraction II. If 2 percent ethanol is present and all the dieldrin still does not elute in Fraction II, the presence of moisture in the system may be the cause. An excess of moisture may result in all or most of the dieldrin eluting in the 6 percent fraction.

c. The activity characteristics of Florisil may vary somewhat from lot to lot. Each lot, when received at a laboratory, should be carefully evaluated to be certain the compound elution characteristics are satisfactory.

d. Storage and holding temperature of Florisil are critical. The oven used for holding this (and other adsorbents) should be confined exclusively to this usage and not used as an all-purpose drying oven. Florisil will readily pick up air-borne contaminants which may result in spurious chromatographic peaks. If the oven temperature varies more than ± 1°C, considerable influence may be observed in the retention characteristics. The recommended activation temperature is 130°C.

e. Anhydrous Na_2SO_4 used to top the Florisil column, even AR grade, frequently contains sufficient impurities to result in spurious peaks in the blank eluates. Because of the prevalence of this situation, it is good practice to Soxhlet extract all lots of this salt before use.

f. The presence of peroxides in ethyl ether can result in extremely low recoveries of organophosphorus compounds and also poses a serious safety hazard. Methods have been set forth for the removal of peroxides from ether but have not proven wholly satisfactory. The purity of petroleum ether is also critical and may exert a profound effect on the recovery of certain of the organophosphorus compounds.

g. Glassware must be meticulously cleaned to remove electron capturing contaminants. Reagent blanks must be run with each set of samples.

h. Most chlorinated pesticides should be recovered in the range of 85-100 percent. HCB is an exception because of an unfavorable partition ratio in the acetonitrile-petroleum ether solvent system. An aldrin spike can be added to the minced fat at the start of the procedure if this pesticide is known to be absent. Recovery of this spike should not be less than 70 percent.

See also Subsection 7K for further comments on pesticide elution from Florisil.

7B HUMAN OR ANIMAL TISSUE AND HUMAN MILK ANALYSIS BY THE MICROMETHOD

If the size of the available tissue sample is so small as to make the macro MOG method unsuitable, a micromethod is described in Section 5,A,(2) of the EPA PAM requiring as little as 200-500 mg of sample. The sample is extracted with acetonitrile, pesticides are partitioned into hexane, fractionated on a 1.6 gram Florisil column (eluate I: 12 ml of hexane plus 12 ml of 1 percent methanol in hexane; eluate II: 12 ml of 1 percent methanol in hexane), and concentrated fractions determined by EC-GC. Several pesticides, including α-BHC, lindane, diazinon, DDD, and toxaphene, split between fractions. Florisil columns must be conditioned at 130°C at least overnight before using. Precautions concerning use of Florisil are similar to those outlined in Subsection 7A. Virtues of the micromethod include a low background level and savings in the volume of solvent required.

7C HUMAN BLOOD OR SERUM

A 2 ml aliquot of serum is extracted with 6 ml hexane for 2 hr on a slow speed rotary mixer. After concentration, the hexane layer is analyzed by EC-GC [EPA PAM, Section 5,A,(3),(a)]. The procedure involves no cleanup, but, if carefully handled, it is capable of yielding recoveries of chlorinated pesticides comparable to that obtained from a full MOG cleanup technique (see Table 2-11 in Section 2). Since all pesticides will be present in one extract, a GC column must be chosen which will separate the expected pesticides. Certain serum samples will yield a very late eluting extraneous peak (probably a phthalate) which is sometimes large enough to distort a following chromatogram if time is not allowed for its elution from the column. Blood samples should never be stored in containers with polyethylene or rubber caps. Hexane was proven superior to hexane-formic acid for extraction of dieldrin, lindane, and DDT from serum (1). Microcoulometric GC determination after sulfuric acid extraction was successfully applied to 24 organochlorine pesticides in blood at 1 ppb levels with no cleanup (2).

Blood samples are not always analyzed without cleanup steps. Monitoring of fur seal blood for OCl pesticides and PCBs required chromatography of the hexane extract on a 2.3 gram Florisil column prior to EC-GC (3). Another monitoring study of pesticides in human blood was carried out by hexane extraction of acidified samples followed by cleanup on a 1 gram Florisil column and EC-GC (4).

7D PENTACHLOROPHENOL (PCP) IN BLOOD AND URINE

Acidified blood is extracted with benzene on a Roto-Rack for 2 hours followed by methylation of PCP and determination by EC-GC (EPA PAM, Section 5,A,(3), (b)). Urine is made alkaline and extracted with hexane to remove basic and neutral interfering compounds (e.g., DDT, BHC), followed by acidification, hexane extraction, methylation, and EC-GC (EPA, PAM, Section 5,A,(4),(a)). The following comments pertain to these methods:

a. The alkylating reagent diazomethane is a hazardous chemical and must be handled with extreme caution.*

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*/ Diazomethane and related alkylating reagents (e.g., diazoethane, diazopentane) have been widely used in pesticide residue analysis and are cited in several procedures in this Manual and the EPA PAM. These compounds and their precursors are toxic and carcinogenic and are irritating to the skin. Solutions have been known to explode inexplicably. It is recommended that safer substitutes be found for these reagents whenever possible, for example BF_3-methanol for methylation of acid herbicides and acetic anhydride for acetylation of pentachlorophenol (Section 7AA). Substitution of one reagent for another, however, can require a large amount of effort to check the validity of the procedure with the new reagent. If diazolkane reagents must be used to reproduce established analytical procedures, take care to keep from direct contact with the skin. Wear disposable vinyl gloves and safety goggles, and avoid breathing of vapors. Work behind a safety shield in an efficient hood or inside a radiological glove box. Do not prepare or store reagents in ground glass stoppered or etched glassware. Avoid strong light.

b. A 1.5% OV-17/1.95% QF-1 column is not recommended since the relative retention values for 2,4-D methyl ester and PCP methyl esters are identical and these pesticides would not be differentiated.

c. All reagents including distilled water must be pre-extracted with hexane to remove interfering materials. Reagent blanks should be carried through the entire procedures with each set of samples and standards.

d. Glassware should be washed with dilute NaOH followed by deionized water and acetone.

e. Contact between wooden or paper materials and glassware should not be permitted as some of these materials have been found to contain significant levels of PCP.

f. Other ether derivatives (e.g., ethyl, propyl, amyl, etc.) can be prepared and characterized for confirmation of PCP identity.

g. A Vortex-Genie or similar mixer should be available for efficient extraction and quenching of the derivatization reaction.

An improved EC-GC method for determination of PCP in urine and blood has been worked out as part of a multi-phenol analytical procedure. A hydrolysis step is included to increase recovery of PCP compared to extraction with hexane only. A brief outline of the full procedure is given below; further details can be obtained from Dr. R. Moseman, U.S.E.P.A., Health Effects Research Laboratory, Research Triangle Park, NC 27711:

a. Acidify 2 ml of urine with 0.5 ml conc. HCl or 1 gram of blood serum with 3 ml of 6N HCl in a 125 mm screw cap culture tube.

b. Seal the tube and place in a boiling water bath for 1 hour with periodic shaking.

c. Extract the cooled sample twice for one-hour periods on a mechanical rotator at 30-50 rpm with 5 ml portions of benzene for urine or with hexane/ethyl ether (1:1) for blood.

d. Centrifuge after each extraction and concentrate the combined extracts to 0.3-0.5 ml.

e. Methylate with 5 ml of diazomethane reagent. (See footnote on page 4 when using diazoalkane reagents.)

f. Allow the methylated extract to stand for one hour and concentrate to 0.3 ml under a gentle stream of nitrogen.

g. Add 2 ml of hexane and reconcentrate to 0.3 ml.

h. Chromatograph the methylated sample through a size 22-9 Kontes Chromaflex column containing 4 grams of acid alumina topped by 1.6 grams of Na_2SO_4. Elute the column with a total of 5 ml of hexane (including the volume - ca. 3 x 0.5 ml - used for rinsing the sample onto the column) and discard eluate; then elute the PCP methyl ester with 20 ml of benzene-hexane (10:90 v/v). (The prepared column is washed with 30 ml of benzene-hexane (40:60 v/v) to remove impurities, air dried, and stored in an oven at 130°C overnight prior to use.)

i. Concentrate the eluate to an appropriate volume and compare to similarly treated PCP standards using EC-GC with standard columns and conditions described in Section 5 of this Manual and Sections 5,A,(3),(b) and 5,A,(4),(a) of the EPA PAM.

7E BIS(p-CHLOROPHENYL)ACETIC ACID (p,p'-DDA) IN HUMAN URINE

The excretion level of this metabolite is a sensitive indicator of exposure to p,p'-DDT. Urine is extracted three times with an equal volume of 2 percent acetic acid in hexane, the combined extracts are evaporated to remove residual water or acetic acid, DDA is converted to its methyl ester by reaction with BF_3-methanol reagent, and the ester is extracted with hexane and determined by GC with microcoulometric or EC detection [EPA PAM, Section 5,A,(4),(b)].

Microcolumn Florisil cleanup (Subsection 7B) is required when the poorly selective EC detector is used. DDA should elute completely in Fraction II. Concentration and injection volumes depend upon the sensitivity of the detector employed. A column of 5% OV-210 at 175-180°C will separate DDA from p,p'-DDE (which usually is also present in high exposure donors) whereas 4% SE-30/6% QF-1 or 1.5% OV-17/1.95% QF-1 columns at 200°C will not resolve these compounds.

7F 2,4-D AND 2,4,5-T IN URINE

A method is described in the EPA PAM, Section 5,A,(4),(c), for determining these herbicides and their degradation products 2,4-dichlorophenol and 2,4,5-trichlorophenol in human and animal urine. Phenolic conjugates are hydrolyzed in acid, and free phenols and acids are extracted with benzene and ethylated with diazoethane. Cleanup and fractionation of derivatives is carried out on a silica gel column (1 gram, containing 1.5 percent water), and determination of concentrated eluates by EC-GC on a 4% SE-30/6% OV-210 column.

Deactivated silica gel (Subsection 4Ad in Section 4) columns should be prepared just prior to use. Because of the differences in temperature and humidity from one laboratory to another, silica gel elution parameters should be established by each analyst under local conditions. The percentage water added for deactivation should be increased if the compounds of interest elute in a later fraction than that indicated in the detailed procedure, or, the percentage of benzene in the benzene-hexane eluent can be increased. Early elution would be remedied by less deactivation or less polar solvents. Spiked control urine, rather than standard compounds, should be used to determine the elution pattern. See the footnote on page 4 concerning the hazards associated with the ethylating reagent. Alkylated standards are stable for one month if stored in a freezer (-18°C) when not in use.

A multiresidue scheme for phenol metabolites and including 2,4-D, 2,4,5-T, and silvex is discussed in Subsection 7O.

7G KEPONE IN HUMAN BLOOD AND ENVIRONMENTAL SAMPLES

The determination of Kepone in human blood, air, river water, bottom sediments, and fish is described in the EPA PAM, Section 5,A,(5),(a). This is based on the research of Moseman et al. (5). Samples are extracted, and the extracts are cleaned up by chromatography on a micro Florisil column, base partitioning, or gel permeation chromatography. Kepone is determined by EC-GC and confirmed by chemical conversion to mirex (6), detection with a Hall conductivity detector in the Cl-mode, or chemical ionization mass spectrometry.

It is mandatory to use 1-2 percent methanol in benzene for all sample and standard solutions injected for EC-GC to obtain the maximum reproducible response. Sufficient control and spiked reference materials should be utilized to ensure the validity of analytical results for all sample types. Elution patterns for the Florisil columns should be carefully established by each analyst by eluting standard Kepone under local laboratory conditions.

The analysis of field-collected avian tissues and eggs for Kepone residues has been reported (7). Samples were extracted with benzene-isopropanol (2:1 v/v) and extracts cleaned up with fuming H_2SO_4-concentrated H_2SO_4 (1:1 v/v). Separation of Kepone from OCl pesticides and PCBs was obtained on a 10 gram 130°C-activated Florisil column eluted with 100 ml of benzene-acetone (95:5 v/v) followed by 200 ml of benzene-methanol (90:10 v/v); the second eluate contained the Kepone. Determination was by EC-GC on a 4% SE-30/6% QF-1 column and confirmation by GC-MS. Recoveries averaged 86 percent at 1 ppm. Procedures for determination of Kepone in serum, plasma, urine and fat have been reported. After addition of H_2SO_4, samples were extracted with hexane-acetone (17:3 v/v), extracts were evaporated, and the residue dissolved in benzene-methanol (99:1 v/v). The extraction was modified for feces and bile. Programmed temperature GC with pulsed EC detection on a 4% SE-30/6% QF-1 column provided linear calibration curves for 10 pg-100 ng of Kepone (5 ppb-50 ppm/gram sample) (8).

7H ANALYSIS OF FATTY AND NONFATTY FOODS BY THE MOG METHOD

The macro Florisil column method for determining nonionic chlorinated pesticides in fatty foods is similar to that outlined in Subsection 7A and is described in detail in Sections 211 and 231 of the FDA PAM. Eluents are 6, 15, and 50 percent ethyl ether in petroleum ether. The method for nonfatty foods (FDA PAM, Sections 212 and 232) involves extraction of pesticides with acetonitrile or water-acetonitrile and partition into petroleum ether prior to Florisil column chromatography and EC-GC. The FDA PAM lists pesticides recovered through these procedures (results for 179 pesticides and other chemicals are given in Table 201-A, and over 200 compounds have been tested (9)), crops to which they are applicable, and supplemental cleanup procedures for the Florisil column fractions. This AOAC multiresidue method is currently official for 25 OCl and OP pesticides and PCBs (9). The problem areas are the same as those given in Subsections 7A and 7K. The elution pattern of more than 150 pesticides from the U.S.F.D.A. Florisil column eluted with 6, 15, 20, 30, 50, and 65 percent diethyl ether in petroleum ether is tabulated in Section 7.2(b) of the Canadian PAM.

In order to obtain more efficient cleanup of extracts of fatty foods and recovery of additional pesticides of higher polarity (e.g., organophosphates), a new elution system consisting of three different mixtures of methylene chloride, hexane, and acetonitrile was devised as replacement for the traditional diethyl ether-petroleum ether eluents. These eluent mixtures are methylene chloride-hexane (20:80 v/v); methylene chloride-acetonitrile-hexane (50:0.35:49.65 v/v); and methylene chloride-acetonitrile-hexane (50:1.5:48.5 v/v). At least 50 pesticides and related chemicals have been recovered, in groupings different from the mixed ether systems, with these new solvents (10). A rapid screening method for residues in milk is another modification of the standard procedure. Pesticides are cleaned-up by partioning, but Florisil chromatography is excluded (11).

7I DETERMINATION OF CHLOROPHENOXY HERBICIDES IN FATTY AND NONFATTY FOODS

Section 221 of the FDA PAM covers the analysis of chlorophenoxy acid herbicides in fatty foods. The pesticides are extracted from the foods, partitioned into alkaline solution, the extract is washed with organic solvents and acidified, and the pesticides are extracted into chloroform, methylated with diazomethane, and determined by microcoulometric GC. The recovery of eight compounds from oils, dairy products, and animal tissues has been verified. The cleanup procedure is not sufficient for determination by EC-GC. Methylated acids are easily lost during evaporation steps, especially from standard solutions. Solvents should be evaporated very carefully in a gentle stream of nitrogen. Traces of soap which are not rinsed away can give interference peaks even with the selective microcoulometric detector.

Chlorophenoxy acids and esters are extracted from nonfatty foods with mixed ethers under acid conditions, extracts are shaken with sodium carbonate solution, and esters are extracted with ether and determined by microcoulometric GC after cleanup on a Florisil column. The carbonate solution is acidified to reform the free acids, which are extracted into chloroform, methylated (see the footnote on page 4 concerning the hazards of diazoalkanes), and determined by microcoulometric GC without column cleanup (FDA PAM, Section 222). The recovery of residues of eight compounds in grains and vegetables has been verified. Sodium sulfate used in drying steps may adsorb some free chlorophenoxy acids, and the reagent should be checked for complete recovery. An alternative alumina and Florisil column cleanup prior to EC-GC for the ester fraction from vegetable samples is given in the Canadian PAM, Section 7.3.

A more recent method for determining the acid herbicides in foods and including Florisil cleanup has been reported (12). Residues are extracted with acetonitrile (nonfatty foods) or chloroform (fatty foods and liquids); the acids are converted to their sodium salts, acidified, methylated, cleaned-up on a Florisil column, and determined by EC- or microcoulometric GC. Severe emulsions form during cleanup of the basic phase by extraction with chloroform and must be broken by centrifugation.

7J CARBON-CELLULOSE COLUMN CLEANUP

Section 7.1 of the Canadian PAM describes a method for cleanup of residues of organochlorine and organophosphorus insecticides, herbicides, and fungicides in foods following acetonitrile extraction (blending) and hexane partition. Sequential elution with three solvents (1.5 percent acetonitrile in hexane, chloroform, and benzene) separates the pesticides into three fractions which are suitable for EC-GC and TLC determination.

Some 40 pesticides have been quantitatively recovered from a variety of foods with this system. A carbon-cellulose (4:10 w/w) minicolumn eluted with ethyl acetate has been used for cleanup and separation of phorate and metabolite residues in crops determined by FPD-GC (13).

The Canadian PAM specifies that carbon (e.g., Darco G60) is prepared by heating for 12 hours at 300°C and then extracting for two one hour periods with hexane on a mechanical shaker. See also Section 4Af in this Manual. Cellulose (e.g., Solka Floc BW40) is extracted twice with acetone in a similar manner without prior heating.

7K CLEANUP ON DEACTIVATED FLORISIL

The method of Osadchuk *et al*. is described in the Canadian PAM, Section 7.2. Deactivated Florisil is prepared as outlined in Subsection 4Ac in Section 4 of this Manual. The elution behavior of over 50 pesticides on Florisil deactivated with 2 percent water has been determined for use after extraction and partition cleanup of residues. A 30 cm x 2.5 cm id column containing 15 cm of adsorbent is eluted with 300 ml portions of the appropriate eluting mixture(s) ranging from pure hexane to 5-30 percent methylene chloride in hexane to 5-30 percent ethyl acetate in hexane (Table 7-1). If the analyst wishes to screen a sample extract for a larger number of pesticides in one or two GC injections, the less polar eluents may be by-passed and only the more polar used. However, some sample types may be inadequately cleaned-up by this procedure or mutually interfering residues may occur in the same fraction.

The following factors affect the success of this Florisil procedure:

a. Pesticides containing a mercaptan function are oxidized on the Florisil column. For example, phorate, captan, carbophenothion, chlorobenside, disulfoton, and demeton have losses ranging from 20-100 percent. The oxidation proceeds to the sulfoxide and then to the sulfone. Therefore, non-detection of such pesticides does not guarantee they were not originally present in the sample. The degree of oxidation by Florisil increases with a lower extent of water deactivation (greater adsorbent activity) or a greater time of contact with the column and may also be affected by the pH of the particular Florisil used.

b. Oxygen analogs of organophosphorus pesticides are strongly adsorbed on Florisil and cannot be completely eluted even with very polar solvents.

c. The 2 percent deactivated Florisil column can tolerate up to one gram of fat or oil (30 percent methylene chloride in hexane or less polar eluents) without extraneous EC-GC response.

d. Up to two grams of fat or oil can be applied directly to the column and eluted with 10 percent methylene chloride in hexane to recover BHC isomers, the DDT group, PCBs and HCB.

Table 7-1

ORDER OF ELUTION OF PESTICIDES FROM FLORISIL PARTIALLY DEACTIVATED WITH 2% WATER USING 300 ml VOLUME OF ELUENTS

(From the Canadian PAM)

PESTICIDES	1	% CH_2Cl_2 in Hexane						% EtOAc in Hexane				Percent Recoveries
		Hexane	5%	10%	15%	20%	30%	5%	10%	20%	30%	
Aroclor 1254	PCB	+										>95
Chlordane		+										>95
Toxaphene		+										>95
Strobane		+										>95
trans-Chlordane		+										>95
Chlordene		+										>95
Aldrin		+										>95
Hexachlorobenzene		+										>95
Heptachlor		+										>95
p,p'-DDE		+										>95
o,p'-DDT		+										>95
Mirex		+										>95
Isobenzan		+[2]										>95
p,p'-DDT		S(90%)	+									>95
α-BHC		S(45%)	+									>95
Perthane		S(10%)	S(85%)	+								>95
p,p'-DDD			+									>95
Chlorbenside	M		+[2]									~60
γ-BHC			S(80%)	+								>95
PCNB			S(80%)	+								>95
TCNB			S(45%)	+[2]								>95
β-BHC			S(10%)	+								>95
δ-BHC					+							>95
Dicofol					+							>95
Ronnel	OP				S(65%)	+						>95
Hepachlor epoxide					S(60%)	+						>95
Dichlofenthion	OP					+						>95
Phorate	OPM					+						~ 20
Carbophenothion	OPM					S(25%)	+					~80
Endosulfan I						S(10%)	+					>95
Dieldrin							+					>95
Chlorpyrifos	OP						+					>95
Endrin							●					>95
Methoxychlor							●					>95
Parathion	OP							+				>95
Ethion[2]	OP							+				>95
2,4-D methyl ester								+				>95
2,4,5-T methyl ester								+				>95
Anilazine								+				>95
Ovex								+				>95
Fenitrothion	OP							S(90%)	+			>95
Tetradifon								S(90%)	+			>95
Diazinon	OP							S(90%)	+			>95
Chlorothalonil								S(70%)	+			>95
Methyl Parathion[3]	OP							S(50%)	+			>95
Sulphenone								S(15%)	+			>95
Dioxathion	OP								+			>95
Malathion	OP								●			>95
Atrazine[4]									+			>90
Simazine[4]									+			>90
Endosulfan II									S(75%)	+		>95
Captan	M									+		~80
Phosmet	OP									+		>95
DCPA										+		>90
Azinphosmethyl	OP										+[2]	>95

Note: A 30% CH_2Cl_2 fraction was eluted prior to all ethyl acetate fractions. All others were single elutions.

+ - mostly elutes in first 250 ml
● - large amount in 250-300 ml fraction
S - some (as percent)

Footnotes: 1 - OP = organophosphorus; M = mercaptan; PCB = polychlorinated biphenyl
2 - Higher recoveries are obtained by elution with more polar eluents
3 - Remaining methyl parathion elutes in another 50 ml of 5% EtOAc
4 - Detected by alkali flame detector

The latter approach with hexane elution has been used to determine as low as 5 ppb of HCB in adipose tissue (ref 14; and Section 12,D,(2) of the EPA PAM). The recovery of HCB and mirex in fish and butterfat by elution with acetonitrile from a column composed of the fat or oil distributed on unactivated Florisil has been collaboratively studied (15). Care must be taken in analyses for HCB not to use plastic wash bottles, since this compound was found as a contaminant in 30 of 34 such bottles tested (16).

7L LOW TEMPERATURE PRECIPITATION

This procedure (Canadian PAM, Section 7.4) is used to separate fats, oils, and water from acetone-benzene-acid extracts of biological samples by precipitation at -78°C. The special low temperature cleanup apparatus is described in detail (Canadian PAM, Section 14.5). Many apolar and polar residues and metabolites (e.g., DDT, 2,4-D acid and ester, parathion, and paraoxon) are retained in the acetone supernate and can be determined by EC-GC. Forty pesticides have been quantitatively (80+ percent) recovered from a variety of plant and animal products at levels greater than 0.05 ppm. Freeze-out has been recently employed for the removal of lipids prior to Florisil chromatography and EC-GC in the determination of methoxychlor residues in microsamples of animal tissues and water at 10 ppb and 1 ppb levels, respectively (17).

7M MISCELLANEOUS MULTIRESIDUE CLEANUP PROCEDURES

Other frequently applied multiresidue procedures include the following: The method of de Faubert Maunder (18) employs partition with dimethylformamide (DMF) to diminish the amount of fat carried over with the pesticides from fatty samples. A hexane extract of the sample is extracted three times with hexane-saturated DMF; the combined DMF phases are washed with a DMF-saturated hexane and then shaken with a large volume of 2 percent Na_2SO_4 solution. On standing, a hexane layer containing chlorinated pesticides forms on top of the solution, this layer is separated, and residues are cleaned-up on an alumina column and determined by EC-GC.

Wood (19) proposed the use of dimethyl sulfoxide (DMSO) as another good solvent for chlorinated pesticides because it dissolves low amounts of oil or fat. The fatty sample is mixed with Celite (1:15 w/w) and packed into a small column, and the pesticides are eluted with DMSO. The eluate is adsorbed directly onto the top of a larger Florisil column and the residues then eluted with hexane from the Florisil.

The de Faubert Maunder and Wood methods have been compared with the standard FDA-AOAC Florisil procedure for analysis of chlorinated pesticides in a variety of foodstuffs (20). No gross general differences were found in results, but one method might be advantageous for a particular sample type.

Table 7-2

ORDER OF ELUTION OF ORGANOCHLORINES FROM DEACTIVATED SILICA GEL ACCORDING TO THE METHOD OF HOLDEN AND MARSDEN [21]

Eluted in order by hexane	Eluted in order by 10% diethyl ether in hexane
Hexachlorobenzene	Endrin
Aldrin	Chlordane
PCBs	*p*,*p*'-DCBP
p,*p*'-DDE	Toxaphene
Heptachlor	*p*,*p*'-TDE
p,*p*'-MDE (DDMU)	Telodrin
o,*p*'-DDT	Heptachlor epoxide
p,*p*'-DDT	α-BHC
	Perthane
	β-BHC
	Kelthane
	γ-BHC
	Dieldrin
	Methoxychlor

Hexane extracts of animal tissues are cleaned-up and prefractionated on narrow bore columns dry-packed with partially deactivated alumina and silica gel by the method of Holden and Marsden (21). The initial alumina column eluted with hexane provides removal of lipids, while the second column affords pre-GLC separation of residues plus further cleanup. Table 7-2 shows the elution order of chlorinated insecticides with hexane and 10 percent diethyl ether-hexane eluents. Alumina is activated at 800°C and silica gel at 150°C before deactivation with 5 percent (w/w) water. Another alumina-silica column scheme (22) was devised for separation of 17 OCl residues in 4 eluates, each containing pesticides separable on a 4% SE-30/6% OV-210 GC column. Microcolumns deactivated with 3-4 percent water were used.

Organochlorine insecticide residues in fatty foodstuffs were determined (23) using a cleanup technique based on a single 22 gram column of activity-4 alumina. Concentrated hexane extracts of samples, containing 0.4-0.5 grams of fat, were transferred to the column, and pesticides were eluted with 150 ml of hexane prior to determination by EC-GC. Recoveries of 15 insecticides from vegetable oil samples spiked at levels of 5-250 µg/kg were between 70-124 percent. Routine determinations were carried out for cyclodienes, BHC isomers, and HCB at the 5-10 µg/kg level and DDT-type compounds at the 20-30 µg/kg level. Results of collaborative studies were reported. If PCBs were present, the column was eluted with 10 ml and then 150 ml of hexane. The first fraction contained all the PCBs and all or most of any residues of aldrin, heptachlor, HCB, p,p'-DDE and o,p'-DDT. The second fraction contained all the BHC isomers, heptachlor epoxide, dieldrin, endrin, p,p'-DDD, methoxychlor, and Endosulfan A. Compounds splitting between fractions included methoxychlor, toxaphene, perthane, chlordane, and strobane.

In a comparative study (24), basic alumina was found to retain lipids better than Florisil, which in turn held more than silicic acid. It was also found that deactivation and elution with less polar solvents gave a superior separation of organochlorine pesticides from lipids than activated adsorbents and more polar eluents. Saponification with ethanolic NaOH followed by alumina column chromatography provided efficient removal of lipids prior to GC determination of several OCl insecticides (DDT was converted to DDE) (25).

A gel permeation chromatography (GPC) system using Bio-Beads S-X2 crosslinked polystyrene gel has been designed by Stalling *et al.* (26) for removal of lipids from extracts of samples such as fish before EC-GC determination of commonly occurring pesticide and PCB residues.

In a typical cleanup, a fatty tissue sample may be desiccated with anhydrous Na_2SO_4 (e.g., 1:3 w/w) and then extracted with 6 percent diethyl ether-petroleum ether. The extract is concentrated, brought to volume with cyclohexane or another suitable solvent, and an aliquot injected onto the GPC column. The lipids, because of their high molecular weights, are excluded from the gel pores and elute in the first fraction which is discarded. The fraction containing the lower molecular weight pesticides and PCBs elutes later and is collected and concentrated for GC analysis. Up to 500 mg of lipid can be injected as a 5 ml aliquot onto the GPC column. An evaluation of this GPC system (27) with different sample types indicated that ca. 98 percent of the fat or oil content of the extract is generally eluted prior to the pesticide fraction and that this cleanup may be superior to that achieved by acetonitrile partition and Florisil adsorption. Analyses can be automated since an important feature of GPC is that the same column can be used repeatedly over long periods without significant change in elution volumes or recoveries. Details of GPC cleanup are presented in Subsection 8M in connection with the quality assurance of tissue analysis by GC-MS.

A rapid DMSO-petroleum ether partitioning cleanup method employing test tubes and syringes in place of separatory funnels was found to recover 60 OCl, OP, and carbamate pesticides at levels >50 percent. Losses were found to be consistent, so the use of correction factors was proposed. Crops containing 0.1-10 ppm levels were tested for analysis by GC (EC and FPD detectors), TLC, and HPLC (28).

A reuseable, macroporous silica gel column provided fractionation and 88-105 percent recoveries of 0.1-1 ppm levels of different classes of pesticides when eluted with a series of solvents of increasing polarity (29).

Thin layer chromatography (TLC) on 1-5 mm layers can provide cleanup if a minimal amount of fatty material is present in the extract. Sample is applied as a streak and developed along with standard marker compounds on the same plate to allow location of the pesticide zones. These bands are removed by scraping and are extracted to recover the separated pesticides. Modified layers have been devised with capability for increased sample loading, e.g., multiband or wedge-layer chromatoplates (30). With the latter, cleanup and determination can be combined on the same layer without intervening elution.

The use of ion exchange resins for cleanup of ionic pesticides has been reviewed (31). For example, acidic residues such as chlorophenols and phenoxy acids in extracts of organic tissues, soil, and water will bind under alkaline conditions to a strong base anion exchange resin. After washing out impurities, the residues can be eluted from the resin column by an acidic eluent and determined by EC-GC after appropriate derivatization reactions (32).

The results of international cooperative studies of OCl pesticide, PCB, and Hg residues in wildlife have been reported (33). The analytical methods were based on extraction, cleanup, and GC determination, but no two laboratories used exactly the same procedure. Nonetheless, there was reasonable agreement among laboratories in analysis of test samples, the coefficient of variation for different chlorinated compounds ranging from 10-17 percent. Collaborative testing of a multiresidue method for chlorinated hydrocarbon and other fumigant residues among 8 foreign laboratories was successfully completed, and results were reported (34).

ORGANOPHOSPHORUS (OP) PESTICIDES

7N DETERMINATION OF METABOLITES OR HYDROLYSIS PRODUCTS IN HUMAN URINE, BLOOD, AND OTHER TISSUES

The determination of alkyl phosphate metabolites in urine provides a measure of the extent of human exposure to the parent OP pesticide. Section 6,A,(2),(a) of the EPA PAM and reference (35) contain a sensitive and selective analytical procedure for alkyl phosphate and phosphonate metabolites (hydrolysis products) of important pesticides.

OP metabolites in urine are extracted quantitatively with an anion-exchange resin after addition of acetone in a 10:1 ratio to precipitate some interfering compounds. The compounds are eluted from the resin, derivatized with diazopentane (see footnote on page 4 for precautions when using this reagent), and the derivatives determined by FPD (P-mode)-GC. If very low levels of alkyl phosphate metabolites are present, further cleanup on a 2.4 gram silica gel column deactivated with 20 percent water is carried out. Confirmation is by FPD-GC using both the P and S detector modes (recall that the S-mode is 5 to 10 times less sensitive). Analysis can be made at the 0.1 ppm level, so that the excretion of alkyl phosphates in urine can be detected at pesticide levels much lower than those which result in cholinesterase inhibition. The general class of organophosphate pesticide (but not the exact compound) involved in the exposure may be deduced by characterizing the metabolite(s) excreted. These analytical methods have been applied to the analysis of the urine of rats exposed to a group of aromatic and aliphatic OP and phosphonate pesticides (36).

Because of the complexity of this method, routine analyses should be validated by simultaneous analysis of spiked SPRM's. As outlined in Section 3, one SPRM is analyzed along with each unknown if only occasional analyses are performed, or the ratio of SPRM to routine analyses is at least 10 percent when larger numbers are involved. Because of the instability of urine samples spiked with alkyl phosphates, large samples of SPRM cannot be prepared ahead of time for periodic analyses. A method for preparation of individual SPRM as needed is detailed in Section 6,A,(2),(a), XI of the EPA PAM.

Underivatized compounds may accumulate on the GC column after periods of extended use. Injection of 1 µl of diazopentane solution should be made every two weeks to react with these compounds. If peaks appear following this injection, the column should be reconditioned (Subsection 4I in Section 4). Further confirmation of any particular metabolite can be accomplished by preparing its hexyl derivative.

In addition to alkyl phosphates, significant amounts of the corresponding mono- and dicarboxylic acids are found in the urine of humans exposed to malathion. A silica gel cleanup FPD-GC method for determining these acids as a measure of exposure to malathion has been devised (37). Urine is extracted, the extract is alkylated, and derivatized carboxylic acids are cleaned up according to a previously published (38) alkyl phosphate method. Additional cleanup by solvent partitioning with ether and silica gel chromatography (elution with benzene followed by ethyl acetate-benzene (10:90 v/v), collected as one fraction) is also employed. Derivatized MCA and DCA are determined on a 4% SE-30/6% QF-1 column at 200°C.

70 DETERMINATION OF p-NITROPHENOL (PNP) IN URINE

Urinary PNP, the phenolic metabolite of ethyl and methyl parathion, EPN, nitrofen, etc., can be measured as an indicator of exposure to these organophosphorus pesticides. A small volume of urine is hydrolyzed with HCl to form free PNP, then made alkaline and cleaned-up by extraction with benzene-ether, and finally re-acidified and extracted with benzene-ether to remove PNP. An aliquot of dried extract is analyzed by EC-GC with on-column conversion of PNP to the volatile trimethylsilyl derivative [EPA PAM, Section 6,A,(2),(b)].

A multiresidue analytical procedure for halo- and nitrophenols from a range of biodegradable pesticides (organophosphates, phenoxy acids, organohalides) is also useful for determining exposure to these pesticides [39]. A one to five ml sample is treated with a 1/5 volume of concentrated hydrochloric acid and the mixture refluxed at 100°C for one hour. The phenols are extracted with diethyl ether, ethylated by reaction with diazoethane, and the ethyl ethers chromatographed on a silica gel column (2 grams, 2 percent water deactivation). (See the footnote on page 4 concerning precautions when using diazoalkanes). Elution with various concentrations of benzene in hexane purifies and fractionates the phenolic ethers, which are finally determined by EC-GC.

Ten phenols, including the pesticides PCP and DNOC, plus the herbicides 2,4-D, 2,4,5-T, and silvex can be determined by this scheme in one sample. All halogenated phenols are eluted with 20 percent benzene-hexane, while nitrophenols and phenoxy acids elute in the 60 and 80 percent fractions. The phenoxy acids are detected intact along with 2,4-dichlorophenol and 2,4,5-trichlorophenol, their potential mammalian metabolites.

A method for the determination of residues of the herbicide DNBP (2-sec-butyl-4,6-dinitrophenol) in feed, blood, urine, feces, and tissues by EC-GC has been devised in the EPA Health Effects Research Laboratory (40). After extraction, the sample is reacted with diazomethane (see footnote on page 4 concerning precautions when using diazoalkane) to produce the methyl ether of DNBP. Cleanup and recovery of the derivative is obtained on acid alumina column eluted with hexane-benzene (40:60 v/v). Average recoveries of greater than 85 percent were obtained from samples fortified at 0.1-30 ppm levels.

7P ANALYSIS OF FATTY AND NONFATTY FOODS USING FLORISIL CLEANUP

The MOG Florisil procedures described in Subsection 7G are adequate for determination of some OP pesticides in fatty and nonfatty foods (FDA PAM, Sections 231 and 232). Malathion and some other OP pesticides require 50 percent diethyl ether-petroleum ether for elution from Florisil. This elution, which must be preceded by elution with the 6 percent and 15 percent eluents, has been found occasionally to be inconsistent. As was mentioned earlier, OP pesticides can be lost through degradation on the Florisil column and during subsequent evaporations, or when water dilution of the acetonitrile extract for residue transfer to petroleum ether is carried out. Recoveries are tested by carrying known amounts of pesticides through the procedure in the absence of crop substrate. Only 23 of 70 OP pesticides and metabolites tested through the MOG procedure were recovered, and not all recoveries were complete. The AOAC has validated the procedure only for carbophenothion, diazinon, ethion, malathion, methyl and ethyl parathion, and ronnel in 18 fruit and vegetable crops (41-43).

Beckman and Garber (44) recommended the solvent series benzene, diethyl ether-benzene (1:2 v/v), acetone, and methanol for elution of Florisil columns. The elution pattern and recovery of 65 OP pesticides were studied, but sample extracts were not tested. This system was later found to be applicable to the determination of methyl and ethyl parathion, malathion, malaoxon, and paraoxon residues in apples and lettuce, although "all-Florisil" columns were not generally recommended as the best choice for cleanup of OP pesticides (45).

A novel use of Florisil was the development of a partition column consisting of acetonitrile on Florisil for the separation of some pesticides from fish, beef, and butter fat (46). The technique was useful for cleanup of pesticides having favorable p-values (Section 8F) in a hexane-acetonitrile system, which included dimethoate, temephos methyl paration, fenitrothion, crufomate, malathion, and parathion.

7Q SWEEP CO-DISTILLATION

Sweep co-distillation has proven to be a simple time saving cleanup technique which eliminates the need for specialized adsorbents and large volumes of purified solvents (42, 47-52). The technique can be used for OCl and OP residues in fruits and vegetables, or fats and oils. The procedural details are different for the two sample types; however, the cleanup principle is essentially the same. The concentrated sample, in an organic solvent, is injected into a heated tube swept with 600 ml N_2/min. Sample extractives remain in the tube while volatilized components are swept into a simple condensing train. After a 30 minute sweep time, the transfer lines are disconnected and condensed pesticides are rinsed with organic solvent into the sample tube. After volume adjustments, the sample may generally be analyzed by GC without further cleanup. If sensitivity levels in the low part per billion range are desired, an auxillary cleanup is recommended. The combination of sweep co-distillation and the micro Florisil column (EPA PAM Section 5,A(2)) has proven to be a thorough cleanup for fat samples.

Figure 7A is a schematic diagram of the apparatus as originally used for cleanup of fruits and vegetables for determination of OP residues. The glass wool-packed tube was placed inside a heated copper tube. A nitrogen sweep of 600 ml/min was used. Two gram aliquots of sample were injected followed by ethyl acetate injections every three minutes. See FDA PAM Section 232.2 for method details.

OCl and OP residues in a variety of edible fats and oils have been determined by a modified version of the sweep co-distillation cleanup system (53, 54). A tube packed with glass wool, sand, and glass beads is operated in a vertical position with the injection port on bottom. The cleanup is effected by the 250^o heat and the nitrogen carrier gas distributing the oil upward through one-half to three-fourths of the glass bead packed column with a percolation type action. Pesticides are volatilized and swept into the collector trap. Recent study of sweep co-distillation of fats has shown that follow-up injections of solvent are not necessary. After initial injection of the sample, the equipment may be left unattended for the 30 min sweep operation.

Figure 7B shows the appearance of the current commercial version of the "Sweep Co-distiller" (Kontes Glass Co., Vineland, NJ). This apparatus permits simultaneous cleanup of four samples with a 30 min sweep time. The 30 cm tube allows efficient cleanup for OCl or OP residues in one gram samples of fats or oils (operated in vertical position at 250^{o}C with 600 ml N_2/min). The tube for fat cleanup may be purchased prepacked, but packing in the laboratory is preferable for consistent tube uniformity. The empty tube may also be prepared for fruit and vegetable cleanup by packing with 15 cm glass wool in the injection end with remaining space filled with glass beads. The oven would be swiveled to a horizontal position for the fruit and vegetable cleanup. Operational parameters for the latter application may be found in the FDA PAM, Section 232.2.

Figure 7-A. Sweep co-distillation apparatus, schematic diagram.

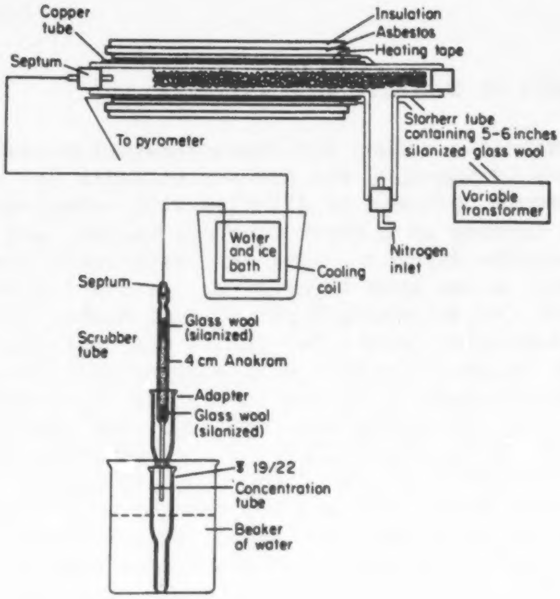
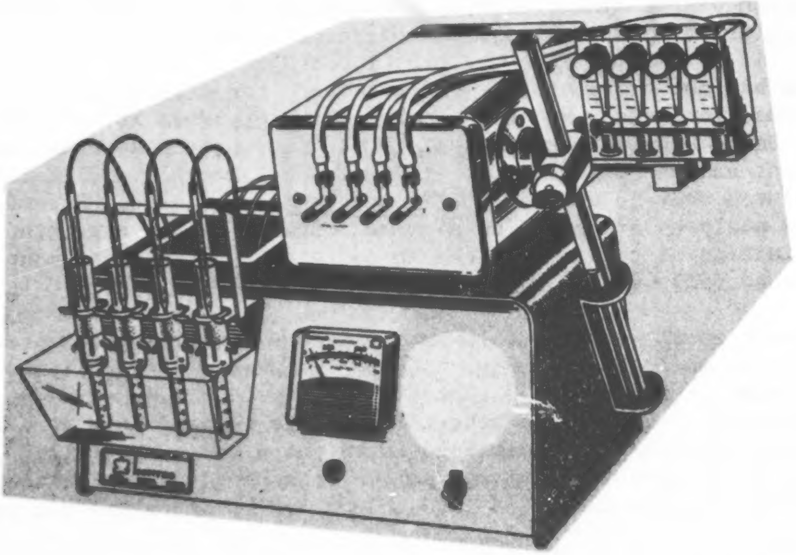

Copper tube
Insulation
Asbestos
Heating tape
Septum
Storherr tube containing 5-6 inches silanized glass wool
To pyrometer
Variable transformer
Water and ice bath
Cooling coil
Nitrogen inlet
Septum
Scrubber tube
Glass wool (silanized)
4 cm Anakrom
Adapter
Glass wool (silanized)
₮ 19/22
Concentration tube
Beaker of water

Figure 7-B. Sweep co-distillation apparatus, (oven positioned for fruit and vegetable cleanup), Kontes Glass Co., K-500750.



7R CHARCOAL CLEANUP OF NONFATTY FOOD EXTRACTS

A general determinative method for organophosphorus pesticide residues in nonfatty foods is based on the FDA acetonitrile (or water/acetonitrile) extraction procedure followed by dilution with methylene chloride to separate water, cleanup on a short charcoal column, and analysis by GC with a P-selective detector. The chromatographic tube (300 mm x 22 mm id) is packed dry with a one gram layer of Celite 545 followed by 6 grams adsorbent mixture (acid-treated Norit SG-X or Nuchar C-190 charcoal-hydrated magnesium oxide-Celite 545, 1:2:4 w/w) and finally glass wool topping, and the column is eluted with acetonitrile-benzene (1:1 v/v). The satisfactory recovery of 41 pesticides and alteration products from kale and 9 typical pesticides from other low and high sugar content crops was demonstrated (55). A collaborative study (56) of this method for residues of six OP compounds in apples and green beans verified recoveries between 86 and 125 percent when either a thermionic or FPD detector was employed. The method is described in the FDA PAM, Section 232.3., and recoveries of 51 pesticides and related chemicals are listed in Table 201-H of the FDA manual. Sections 4Ae and f of this Manual describe procedures for purification of Celite and carbon adsorbents.

7S MISCELLANEOUS MULTIRESIDUE CLEANUP PROCEDURES

Nine extraction procedures were compared for efficiency of removal of six OP pesticides and metabolites from field treated crops. Soxhlet extraction of the finely chopped crops with chloroform-10 percent methanol proved most reliable and efficient (57).

Alumina has not proven totally satisfactory for cleanup of OP compounds since recovery of the more polar compounds is not complete (58). Using alumina (activity II to III) and petroleum ether and 3 percent acetone-petroleum ether as eluents, Renvall and Akerblom (59) eluted only 13 of the 31 OP compounds they tested. However, many residue analyses are based on alumina column cleanup, e.g., the determination of carbophenothion in goose tissues (60) and monocrotophos in tobacco (61) by FPD-GC.

The Abbott et al. method (62), involving cleanup by solvent partition without column chromatography, has proven adequate for analyses of seven types of foods for 39 pesticides and metabolites when detection was made with a thermionic detector. Finely chopped sample is mixed with anhydrous sodium sulfate and extracted with acetonitrile. The extract is diluted with a large volume of aqueous sodium sulfate, and the pesticides are extracted into chloroform. The chloroform solution is dried and concentrated for GC. Other determinations without column cleanup have been reported. Methyl parathion, diazinon, malathion, and phorate were determined in plant, animal, water, and soil samples by EC-GC following only hexane extraction and partition with aqueous acetonitrile (63). Azinphosmethyl and dimethoate residues in apple leaves were determined by FPD-GC following ethyl acetate extraction and cleanup by methylene chloride-water and hexane-acetonitrile partitionings (64). A multiresidue analysis of 14 pesticides in natural waters at ppb levels involving extraction and concentration before FPD-GC has been reported (65).

The elution pattern of a series of representative OP pesticides from a column (Kontes, Size 22) containing one gram Woelm silica gel deactivated with 1.5 percent water and prewashed with 8 ml hexane before applying the sample mixture is as follows:

Eluent	Pesticides Eluted
7 ml hexane	-----
8 ml 60% benzene-hexane	carbophenothion
8 ml benzene	ethyl parathion
8 ml 8% ethyl acetate-benzene	malathion, diazinon
8 ml 50% ethyl acetate-benzene	paraoxon

Silica gel or silicic acid columns have been used for cleanup of animal, plant, soil, and water extracts prior to GC determination of OP pesticides (66-68) and to separate OP pesticides and metabolites into groups to facilitate their identification by GC (69). A tandem column of silica gel and alumina was used to separate leptophos and its oxygen and 2,5-dichlorophenol analogs prior to determination by FPD-GC (70).

The cleanup of 22 OP pesticides in 12 vegetable extracts was achieved by gel filtration chromatography on Sephadex LH-20. Residues were determined by GC with thermionic detection at levels of 0.05 to 0.5 ppm (71). Recoveries ranging from 88-106 percent were reported for disulfoton, diazinon, methyl parathion, malathion, parathion, dichlorvos, and fensulfothion in an evaluation study of the automated gel permeation chromatographic cleanup technique (Section 7M). Biobeads S-X3 gel and a toluene-ethyl acetate (1:3 v/v) elution solvent were used (72). A solvent composed of methylene chloride-cyclohexane (15:85 v/v) with Biobeads S-X3 provided adequate cleanup for EC-GC (no liquid-liquid partitioning) of 9 OP pesticides and 2 metabolites and 16 nonionic OCl pesticides in vegetable oils at 0.05-1.0 ppm. Elution characteristics of 12 other OP compounds, 12 carbamates, 2 triazines, and trifluralin were also investigated (73).

A rapid, simple approach has been developed for approximating the total residues of pesticides such as fenthion, disulfoton, and phorate, which may consist of the parent pesticide and up to five metabolites formed by oxidation of thionophosphate and sulfide groups in each molecule. The insecticides and any metabolites are oxidized to the oxygen analog sulfone with m-chloroperbenzoic acid, followed by removal of the acid on an alumina column and determination of the sulfone by FPD-GC. Quantitative recoveries of parent pesticides and metabolites from corn, milk, grass, and feces have been demonstrated [74]. Metasystox-R and its sulfone were determined in plant and animal tissues and water at 10 ppb levels as the sulfone after oxidation by $KMnO_4$ (75).

A collaborative study by 12 laboratories of the Abbott et al. (62; see above, this Subsection), Watts et al., and Sissions and Telling methods was conducted for OP pesticides in fruits and vegetables (76). The Abbott et al. method was found satisfactory for determination of malathion, dichlorvos, dimethoate, omethoate, and parathion in 6 fruit and vegetable crops (>90 percent average for all pesticides and crops at 0.5-2 mg/kg) and was judged widely applicable to the determination of many other nonpolar and medium-polarity OP pesticides and to a wider range of samples. The Watts et al. method (77), involving ethyl acetate extraction and cleanup on a column of activated charcoal-magnesium oxide-Celite eluted with ethyl acetate-acetone-toluene (an early version of the procedure described in Section 7R), was found satisfactory for the same pesticides plus azinphosmethyl in 6 crops (>90 percent average recovery at 0.5-2 mg/kg) and was also judged to be much more widely applicable. The Sissions and Telling method (78), employing cleanup by batch addition of charcoal followed by hexane and hexane-acetone (98:2 v/v) elution through an activity-5 alumina column was not successful for the more polar pesticides studied. Details and modifications of these methods are discussed in the report of the collaborative study.

A tabulation has been made (79) of the validated applicability of five multiresidue analytical procedures to the determination of some 50 OP insecticides, acaricides, and nematocides. These procedures were the AOAC (11th ed.) 29.001-29.027 general Florisil cleanup method for OCl and OP pesticides; the AOAC (11th ed.) 29.028-29.033 multiple residue carbon column cleanup method for OP pesticides; the AOAC (11th ed.) 29.034-29.038 single sweep oscillographic polarographic confirmatory method; the Abbott et al. method for total diet studies (62); and an undescribed German procedure (80). In addition, individual determinations of some of the compounds by other special methods were reviewed. It was stated that, in general, the multiresidue methods were not usually suitable for metabolites, requiring separate analysis for the parent and metabolite; each method should be compound validated in the worker's own laboratory; and that differences in results were more likely to arise from sampling problems than from the analytical methods themselves.

CARBAMATE PESTICIDES AND MISCELLANEOUS HERBICIDES

7T 1-NAPHTHOL IN URINE

Humans exposed to the N-methyl carbamate insecticide carbaryl excrete in urine relatively large quantities of the metabolite 1-naphthol conjugated as either the sulfate or glucuronide. Determination of 1-naphthol is made by subjecting 5 ml of urine to acid hydrolysis under reflux to break conjugates, extracting the 1-naphthol with benzene, and derivatizing with chloroacetic anhydride solution. After cleanup on a small silica gel column (1 gram, 1.5 percent water), the derivative is quantitated by EC-GC against a peak from standard 1-naphthol similarly derivatized. Details are found in Section 7,A of the EPA PAM.

Elution patterns from the silica gel column must be established at the temperature and humidity conditions prevalent in each laboratory. Spiked control urine treated in the same manner as routine samples is used for this purpose. Traces of water can affect the derivatization reaction and must be avoided. Derivatized standards are stable for about 6 months if stored in a refrigerator.

7U DETERMINATION OF N-METHYLCARBAMATE INSECTICIDES IN BLOOD SERUM AND HUMAN FAT

An unpublished procedure developed in the Methods Development Section of the Analytical Chemistry Branch, EPA, Health Effects Research Laboratory, Research Triangle Park, N.C., simultaneously determines 0.04 to 1 ppm of propoxur, Landrin, carbofuran, aminocarb, mexacarbate, carbaryl, and methiocarb in human serum and fat. The pesticides are extracted with acetonitrile, partitioned into methylene chloride, and cleaned-up by gel permeation and adsorption (silica gel or charcoal) column chromatography. The purified, intact carbamates are derivatized with pentafluoropropionic anhydride and determined by EC-GC. Details are available from the above address.

7V ANALYSIS OF AMINE METABOLITES IN URINE

A method for determination of amine metabolites from anilide, urea, and carbamate pesticides was developed in the EPA Research Triangle Park laboratories (81). Pentafluoropropionic anhydride was the preferred derivatization reagent for the aniline compounds, with cleanup on 1 gram deactivated (3 percent water) silica gel columns. Determination was by EC-GC on a 3% OV-1 column. Recoveries ranged from 85-90 percent at 1.0 and 0.1 ppm.

7W OTHER INDIRECT (DERIVATIZATION) METHODS OF ANALYSIS

Numerous derivatization methods have been used for the indirect measurement of residue levels of parent carbamate insecticides in a variety of agricultural crops and other substrates. These have involved derivatization of the amine or phenol moieties of the pesticides after hydrolysis, or, less often, the intact insecticide. These derivative methods include reaction of intact insecticides with bromine, silylating reagents, acetic anhydride, and trifluoroacetic anhydride. Phenols resulting from alkaline hydrolysis of the parent insecticides have been reacted with bromine (with or without simultaneous esterification), silylating reagents, mono- and trichloroacetyl chloride, pentafluorobenzyl bromide, and 1-fluoro-2,4-dinitrobenzene. The latter reagent is used for derivatization of carbamate insecticides in the methods for air analysis (Section 7Z) and water analysis (Section 7AA) discussed in this Manual and described in detail in the EPA PAM.

Amine hydrolysis products of carbamate insecticides have been reacted with 1-fluoro-2,4-dinitrobenzene and 4-bromobenzoyl chloride. These and other reactions have been surveyed in a review article (82) in which pertinent references are given.

GC methods for phenyl substituted urea and carbamate herbicides are usually based on hydrolysis followed by determination of the corresponding aniline. Anilines have been derivatized with halogen, 4-chloro-α,α,α-trifluoro-3,5-dinitrotoluene, 1-fluoro-2,4-dinitrobenzene, and pentafluoropropionic anhydride. These reactions are also reviewed in reference (82).

A fluorogenic labeling derivatization reaction with dansyl chloride has been combined with HPLC for the determination of N-methylcarbamate insecticides in soil and water. No preliminary cleanup was required, and detection limits were 1-10 ng/4 µl injection (83).

7X DIRECT METHODS OF ANALYSIS

Determinations of intact, underivatized N-methylcarbamate insecticides are hampered by their decomposition on GC columns under ordinary operating conditions (84). Losses can be minimized by the use of specially prepared, conditioned, and maintained columns. Presilanized supports do not provide sufficient deactivation to prevent degradation of carbamates so it is necessary to employ in situ silanization both during initial conditioning and thereafter to restore column performance. Examples of direct analyses of crop extracts include a multiresidue method (85) on 5–6% DC-200 after acetonitrile partition and charcoal cleanup as for OP pesticides (55), determination of carbofuran and other carbamates on 20% SE-30 (86), and determination of 0.2-15 ng of carbaryl and 1-naphthol on a short column of 3% SE-30 (87). Highly deactivated GC column packing prepared according to Aue (88) from acid washed Chromosorb W support, surface modified with Carbowax 20M, has also been successfully used for chromatography of intact N-methylcarbamates without degradation on the column (89). Such columns are extremely promising for performing analyses without required derivatization.

Urea and N-arylcarbamate herbicides are, in general, more thermally stable than carbamate insecticides, and are therefore more amenable to direct determination by GC. For example, columns of 5% E-301 methyl silicone at 150°C (90), 10% DC-200/15% QF-1 (1:1) at 160°C (91), and 5 and 10% DC-200 (92) have been successfully used, the former for multiresidues of urea herbicides and the latter two for carbamate herbicides in foods. However, decomposition of compounds of these types has been noted under certain conditions, and determinations are therefore often made via thermally stable derivatives of hydrolysis products as mentioned earlier.

7Y ANALYSIS OF PLANT AND FOOD MATERIALS

Extraction of urea and carbamate pesticides from plant materials usually involves blending with methylene chloride, acetone, chloroform, acetonitrile, or an alcohol (or these solvents plus anhydrous Na_2SO_4). If the presence of conjugates of hydroxy metabolites is suspected, hydrolysis with an acid during extraction may be included (Section 7A,I).

Cleanup steps include solvent partition and/or liquid column chromatography, the exact nature of which are pesticide- and sample-dependent. For example, a column of 4:1 MgO-cellulose was used for cleanup of carbamate herbicide residues from a variety of foods (91), while Florisil was employed after acetonitrile-petroleum ether partition for the multiresidue, multiclass determination of carbamate, urea, and amide residues (93). Methods for extraction, cleanup, and GC of carbamates, ureas, and other classes of herbicides (triazines, uracils, phenols) have been reviewed (94-96). A multiresidue method for twelve triazine herbicides in crops, water, and soils involving methanol extraction, alumina column cleanup, and gas chromatography with a Carbowax column and thermionic, microcoulometric, FPD, and electrolytic conductivity detectors has been reported (97). Residues of 15 organonitrogen herbicides and fungicides were screened in foods by acetone extraction, partition and Florisil (2 percent water) column cleanup, and CCD-GC determination (98). Herbicides of different types were determined in crops at tolerance levels with no column cleanup prior to GC with N- and Cl-mode conductivity detection (99).

Total residues of Mesurol and its sulfoxide and sulfone metabolites in plant and animal tissues were determined by oxidation of the extract with $KMnO_4$ to convert all residues to the corresponding sulfone, which was detected at a limit of 0.03 ppm by an S-mode FPD (100).

7Z AIR ANALYSIS

Section 8,B of the EPA PAM contains details of a multiresidue, multiclass analytical method for chlorinated, organophosphorus, and N-methylcarbamate insecticides in ethylene glycol trapping solvent from air sampling impingers (Subsection 6,H) (101). Although the method has been tested with only a limited number of representative compounds present in ethylene glycol, it is likely to be suitable for the determination not only of these but of many other pesticides if they can be efficiently trapped by this solvent.

The procedure involves addition of 600 ml of 2 percent sodium sulfate solution to 100 ml ethylene glycol (equivalent to 20 m^3 of sampled air), extraction of pesticides with 40 ml methylene chloride, and fractionation and cleanup by elution through a one gram silica gel (20 percent water) column with 10 ml hexane, 15 ml 60 percent benzene in hexane, and 15 ml 5 percent acetonitrile in benzene. Chlorinated pesticides (found in fractions I and II) are determined by EC-GC, OPs (fractions II and III) by FPD-GC, and carbamates (fraction III) by EC-GC after derivatization with pentafluoropropionic anhydride or 1-fluro-2,4-dinitrobenzene. Recoveries of 90-110 percent of most of the 25 compounds studied were obtained at <1-4 ng/m^3 levels. Problems were encountered with four compounds at low levels because of background interference from the ethylene glycol (Table 1, Section 8,B, EPA PAM). While recoveries of these 25 pesticides from ethylene glycol are satisfactory, it does not follow they will all be efficiently trapped by this medium. In fact, collection efficiencies for volatile organochlorines are known to be poor, and there are no data as yet on carbamates. All indications are that carbamates will present a greater air sampling problem than volatile OCl's.

This new multiresidue approach should be adaptable for the determination of pesticide residues in samples other than air, although additional cleanup steps may be necessary for difficult samples (e.g., gel permeation chromatography for human fat). This general applicability was indicated by the fact that all the pesticides studied were quantitatively extractable by methylene chloride from acetonitrile, which is a common solvent for extractions from human and environmental samples. The application of this method to the determination of 87 pesticides in water is described directly below in Section 7A,A where quality control aspects are discussed.

The previous air analysis method in Section 8,B of the EPA PAM that was replaced by the multiclass, multiresidue method is still being used for monitoring of certain OCl and OP pesticides that are known to be effectively recovered (e.g., 102). This former method, which includes Florisil column cleanup and GC with EC and FPD detectors, was the basis of the former EPA National Air Pesticide Monitoring Programs (102a). Details can be found in earlier editions of the EPA PAM, but use of the new, more widely applicable procedure described above is generally recommended.

7A,A WATER ANALYSIS

A broadly applicable multiresidue, multiclass method for the monitoring of water samples for pesticides is presented in Section 10,A, of the EPA PAM. Recovery studies were conducted on 42 halogenated compounds, 38 OP compounds, and 7 carbamates. Recoveries of >80 percent were achieved for 58 of the 87 compounds, 60-80 percent recovery for 13 compounds, and <60 percent for the remaining 16 compounds (concentration levels 0.09-400 ppb). Pesticides are extracted from water with methylene chloride, and the concentrated extract is chromatographed on a 1 gram deactivated (20 percent water) silica gel column with four different solvents of increasing polarity to separate the pesticides into groups. OCl compounds are determined by EC-GC, OP compounds by FPD-GC, and carbamates by EC-GC after conversion to 2,4-dinitrophenyl ether derivatives. Low recoveries were in most cases traced to losses during the silica gel chromatography step. Evaporation of solutions by air blowdown should not be used because losses of all three classes of pesticides may occur. Concentrations are carried out under a gentle stream of nitrogen. It is important to apply the concentrated extract to the silica gel column at the exact moment the last of the hexane prewash reaches the top surface of the column. The total 0.5 ml extract plus the 1.0 ml hexane rinse must be transferred to the column without loss to minimize the recovery error. Solvents contained in several eluate fractions from the silica gel column may interfere in the GC and carbamate derivatization steps. It is critical to follow the directions for solvent removal and exchange outlined in Section 10,A of the EPA PAM. A number of the OP compounds require considerable column preconditioning by repetitive injection of high-concentration standards in order to obtain linearity of response and accurate quantitation. Sufficient silica gel should be activated (at 175°C) to provide only a one-week supply, and deactivation should be carried out only on the amount required for a 2 or 3 day period. Larger storage periods may result in a shift of the pesticide elution pattern of the final deactivated columns. Each lot of silica gel should be tested for the proper elution pattern with representative pesticide standards eluting in each fraction. Confirmation of pesticide identity should be made by several techniques outlined in Section 8.

Section 10,B of the EPA PAM describes the determination of some free acid herbicides (e.g., MCPA, 2,4-D, 2,4,5-T) in water. The water is adjusted to pH 3 and extracted with methylene chloride. The extract is taken to dryness, pesticides are esterified with 10 percent BCl_3 in 2-chloroethanol, and the resulting esters are extracted with hexane, concentrated, and determined by EC-GC. If cleanup is required, chromatography on silica gel deactivated with 20 percent water is employed. This procedure is a further extension of the multiclass, multiresidue procedure described directly above. When preparing the BCl_3-chloroethanol esterification reagent, work in an efficient exhaust hood and wear disposable vinyl gloves because 2-chloroethanol is toxic by dermal contact or when inhaled.

The reagent is stable for at least thirty days if kept stoppered and refrigerated. As usual, spiked reference material containing the same pesticides at comparable concentrations as in the sample (if these are known) should be analyzed in parallel. Other aspects of quality control are as discussed in the preceding paragraph. BCl_3-methanol was also chosen in another study (103) as the best derivatization reagent for the determination of 8 phenylalkanoic acid herbicides in water (0.01-2.5 μg/L); solvent partition and silica gel (5 percent water deactivated) minicolumn cleanup and EC-GC with an OV-17/QF-1 column were employed.

The Manual of Analytical Methods of the Inland Waters Directorate, Water Quality Branch, Environment Canada, Ottawa, contains detailed methods for the analysis of organochlorinated pesticides and PCBs, organophosphorus pesticides (two procedures), phenoxy acid herbicides, and pentachlorophenol in waters. The method for organochlorines, employing Florisil column cleanup and EC-GC, has detection limits ranging from 0.005-0.1 ppb. The first OP procedure determines dimethoate, fenitrothion, and phosphamidon and the second determines 14 other OP pesticides, all at 0.01-0.1 ppb levels by FPD-GC without cleanup. Phenoxy acid herbicides are extracted with chloroform from acidified water and converted to their methyl esters utilizing BF_3-methanol prior to cleanup on a Florisil column 2 cm in height and EC-GC determination at 0.01-0.05 ppb levels. PCP is detected at 0.01 μg/L by benzene extraction from acidified water, partition into potassium carbonate solution, acetylation with acetic anhydride, partition into hexane, and EC-GC.

Cleanup is often not required for EC-GC analysis of surface water samples (104) and is usually not required for any type of water if a selective GC detector is employed. For example, the multiresidue analysis of 14 OP pesticides in natural waters has been carried out at ppb levels by extraction, concentration, and direct GC with a FPD detector in the P-and S-modes (65). Results of an interlaboratory study of the analysis of 15 water samples for 10 OCl pesticides without any column cleanup have been reported (105). Where needed, cleanup and separation of common chlorinated and OP insecticides extracted from water have been successfully carried out in silica gel microcolumns (106-107) and columns of deactivated (5-20 percent H_2O) silica gel (above) and alumina (108).

Extracts of water, sediment, sludge, sewage, and soil often contain large amounts of elemental sulfur, which interfere in the GC analysis of early eluting pesticides with the EC or FPD detectors. Chemical desulfurization with Raney copper powder (109) or copper ribbon (110), precipitation with metallic mercury (111), reaction with CN^- (112), and treatment with tetrabutylammonium sulfite to produce an ion pair with sulfur as $S_2O_3{}^{=}$ (113) have been used to remove such interference. (See also Subsection 7A,B).

Polar phosphorus, urea, and carbamate pesticides are extracted from water with more polar solvents such as chloroform, acetone, or methylene chloride. Extraction of acidic or basic compounds is aided by adjusting the water sample to a controlled pH value. Determination by GC is carried out using an appropriate selective detector after extract concentration and any required cleanup and/or derivatization steps. As an example, carbaryl and 1-naphthol have been determined in natural water at 2.5-10 ppb levels (82-102 percent recovery at 5 ppb) by EC-GC after methylene chloride extraction, cleanup on an XAD-8 column, and derivatization with HFBA reagent (114).

Chlorophenoxy herbicides and their esters have been determined by adjusting the water sample to pH 2, extracting with benzene or diethyl ether, methylating the acids with diazomethane or BF_3-methanol, followed by gas chromatography with an electron capture or microcoulometric detector (115) (see the footnote on page 4 concerning the hazards of diazomethane).

TLC determinations of carbamate, urea, triazine, and uracil herbicide residues in water have been reviewed (82, 116), as have the extraction, cleanup, GC determination, and confirmation of chlorinated insecticides in water and soils (117).

7A,B SOIL, HOUSE DUST, AND BOTTOM SEDIMENT

The analysis of soil and house dust for organochlorine pesticides is described in Section 11,A of the EPA PAM. Homogenized samples are Soxhlet-extracted with acetone-hexane, extract is concentrated in a K-D evaporator, and cleanup carried out on successive aluminum oxide and Florisil columns. Eluates are concentrated as required and determined by EC-GC. A similar AOAC method has been declared official final action for residues of aldrin, p,p'-DDE, p,p'-DDT, o,p'-DDT, p,p'-TDE, dieldrin, endrin, heptachlor, heptachlor epoxide, and lindane (118).

Sediment samples are partially air dried, mixed with sodium sulfate, and packed into a chromatographic column. The pesticides are extracted from the column by elution with hexane-acetone (1:1 v/v). The extract is washed with water to remove acetone, and the pesticides extracted from water with 15 percent methylene chloride in hexane. The extract is dried with sodium sulfate, concentrated to a suitable volume, and cleaned-up on a Florisil column. After desulfurization with copper, determination of organochlorine pesticides is by EC-GC. Details of the entire procedure are presented in Section 11,B of the EPA PAM.

Air drying of the sample required 1-3 days, depending on the soil type. Such samples will contain at least 50 percent water. Pesticide concentrations are expressed on a "dry" basis, requiring determination of the dry weight of sediment by weighing a separate, air-dried sample before and after heating overnight at 100-110°C. Storage of soils in light can cause formation of artifacts of OCl pesticides (119).

Sediment samples may contain elemental sulfur which will be recovered through the normal extraction and cleanup procedures for organochlorine and organophosphate pesticides and detected by the EC, FPD (P or S modes), and conductivity detectors. With the recommended GC columns and operating parameters, sulfur can completely mask the chromatogram from the solvent peak through the aldrin peak. The technique described in Section 11,B,VI of the EPA PAM for desulfurization employs vigorous agitation for one minute with bright metallic copper. Some pesticides may be degraded by this treatment (e.g., OPs, heptachlor), but these are not likely to be found in routine sediment samples because of breakdown in the aquatic environment. The procedure should be carried out if the presence of sulfur is indicated by an exploratory injection from the final extract concentrate or if sulfur crystallizes out when the 6 and 15 percent ethyl ether eluates from the Florisil column are concentrated.

Nine chlorinated insecticides were determined by a modified GC procedure (120) with recoveries of 75-99 percent from suspended sediment and bottom material. Extraction was with acetone and hexane added separately, coextractives (including PCBs) were isolated by alumina and silica gel column chromatography, and EC-GC was used to analyze the various column eluates.

Shake (blending), Soxhlet, and column extraction methods were compared for efficiency in removing some twenty chlorinated insecticides from a sandy loam soil. There was no statistical difference among the three methods for the majority of pesticides, but shake extraction was significantly more efficient for BHC isomers (121). The shake extraction method with hexane-acetone after moistening the soil with 0.2 M NH_4Cl was studied collaboratively using standard AOAC analytical methods (Florisil cleanup and EC-GC) (122) and found to give excellent recoveries for six insecticides in three different soils (123). Some soil analyses have been carried out by EC-GC with no required column cleanup (124), but this is not common.

Soil residues of chlorfenvinphos, Chlormephos, disulfoton, phorate, and pirimiphos-ethyl were determined by GC with thermionic detection. Extracted compounds were cleaned-up on a carbon-cellulose column. Recoveries ranged from 95-101 percent (125).

A multiresidue GC procedure for the herbicides dichlobenil, dinitramine, triallate, and trifluralin in soils was described by Smith (126). Extraction was carried out with acetonitrile-water (9:1 v/v) in a Sonic Dismembrator, herbicides were partitioned into hexane, and aliquots injected directly into an EC chromatograph. Recoveries were 92-107 percent from three soils at 0.05-0.5 ppm levels. Acetonitrile-water mixtures have proven to be especially efficient solvents for residues of herbicides of different chemical classes (127). Anilide herbicides were determined by GC after extraction from soil by blending with acetone (128). Urea and carbamate herbicides were recovered from soils by shaking with methanol (129) or acetone (130) and by alkaline hydrolysis and steam distillation (131). Iodinated (131) and 2,4-dinitrophenyl (130) derivatives were used for EC-GC determination of the herbicides. Triazines were extracted with diethyl ether from soil treated with ammonia (132) and uracils with 1.5 N NaOH (133). Nineteen acidic, neutral and basic herbicides have been determined in soils by two dimensional TLC (134). Carbofuran residues in soil were determined at the 0.1 mg/kg level without cleanup by EC-GC after ammonium acetate extraction and formation of the dinitrophenyl-ether derivative (135). Uracils have been recovered by elution with water from a column prepared by mixing soil with Celite and $Ca(OH)_2$; the eluate was acidified and extracted with $CHCl_3$, and uracil determination was by RbCl thermionic-GC (136).

The electrolytic conductivity detector has been used to determine nitrogen-containing residues in crude soil extracts. A detector maintenance program for decontamination of the transfer lines and vent valve provided reliable operation with little "down time" even though lengthy extract cleanup was not carried out (137).

The analysis of pesticides of many classes in soils and plants has been reviewed (138).

POLYCHLORINATED BIPHENYLS (PCBs)

7A,C PESTICIDE-PCB MIXTURES

PCBs are among the most ubiquitous and persistent chlorinated pollutants found today in the environment. The residue analyst is concerned not only with the detection and quantitative estimation of PCBs but with their effect on the reliable determination of pesticide residues. PCB interference may occur with most common chlorinated pesticides in residue analysis, and the residue chemist must be aware of the nature of this interference with respect to the GC columns being used and their operating parameters. Interference in routine analysis is possible with *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE, as well as with early eluting pesticides such as BHC isomers, aldrin, heptachlor, and heptachlor epoxide, since prominent PCB peaks have retention times similar to these pesticides on the recommended GC columns.

PCBs are frequently detected in human adipose tissue, often at concentrations similar to those of chlorinated pesticides. Occasionally, however, PCB interference is significant in human tissue analysis, depending upon the columns and operating parameters used. These interferences demonstrate the non-specificity of the electron capture GC detector and the need for careful confirmation by use of at least two GC columns, TLC, chemical reactions, etc. (Section 8).

7A,D APPEARANCE OF PCB CHROMATOGRAMS

Whenever an analyst observes a conglomerate of chromatographic peaks upon injection of a biological substrate into an EC detection system, the possibility of the presence of PCBs should be considered. For example, Figure 7-C shows a chromatogram resulting from the injection of 10 ng Aroclor 1254 on a 4% SE-30/6% QF-1 column operated at 200°C with a carrier flow of 70 ml/minute. The first isomer peak of consequence has an absolute retention of about 6 minutes and the final peak about 38 minutes. Figure 7-D represents the chromatogram of 6 ng Aroclor 1260 under the same conditions, and major peaks ranging from 8 minutes to nearly one hour are seen. Aroclors 1254 and 1260 have shown up most widely in a variety of environmental and tissue samples.

The type of confusion evident when pesticides and PCBs are present in the same substrate is illustrated in Figure 7-E showing Aroclor 1254 co-chromatographed with a mixture of eight chlorinated insecticides. Aldrin (peak 1), p,p'-DDE (3), p,p'-DDD (5), p,p'-DDT (6), Dilan I (7), and methoxychlor (7) are seen to overlap PCB peaks so closely that differentiation would be impossible. Heptachlor epoxide (2) and dieldrin (4) (in large quantities) are partially separated, while Dilan II is fairly well separated. A co-chromatogram of Aroclor 1260 with the same pesticide mixture would show good separation of aldrin and Dilan II, partial separation of heptachlor epoxide and Dilan I or methoxychlor, and complete overlap of DDE, dieldrin, DDD, and DDT.

Figure 7-C. Aroclor 1254. Column
4% SE-30/6% QF-1, 200°C,
carrier flow 70 ml/min.

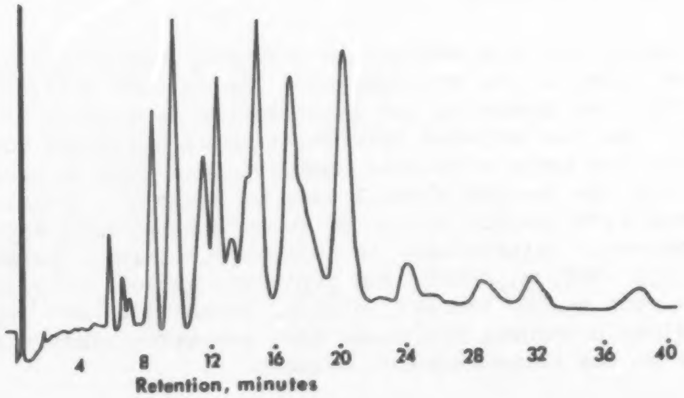


Figure 7-D. Aroclor 1260. Column 4% SE-20/6% QF-1, 200°C, carrier flow 70 ml/min.

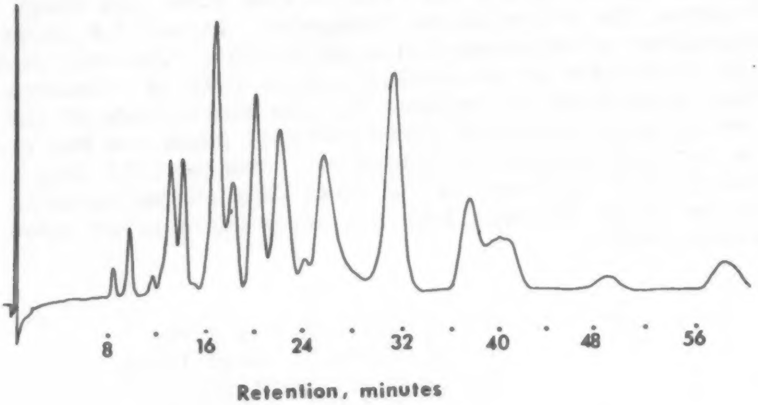
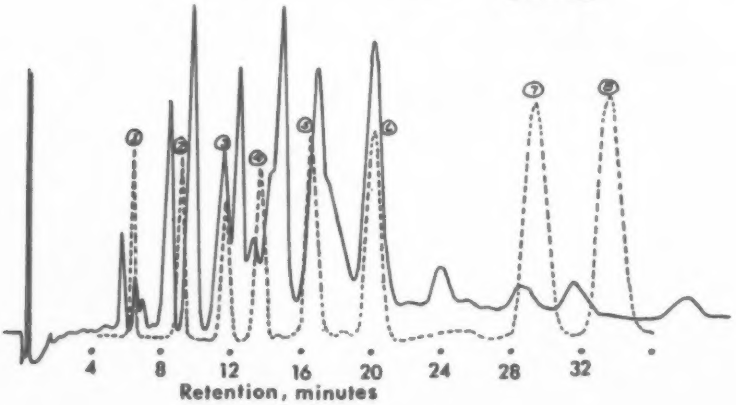


Figure 7-E. Aroclor 1254 (solid line) and pesticide mixture (dotted line). Column 4% SE-30/6% QF-1, 200°C, carrier flow 70 ml/min.



Confusing chromatograms also result when PCBs are mixed with the multi-peak pesticides chlordane or toxaphene. Figure 7-F shows the co-chromatogram of chlordane and Aroclor 1254. The only clean separation is the first peak of the earliest major pair of chlordane peaks, while partial separation is obtained for the second peak of the third pair. The early minor chlordane peaks are well separated but are of little value for quantitation of chlordane. Aroclor 1260 does not interfere as seriously with chlordane under these same chromatographic parameters since the first PCB peak does not elute until after first two major chlordane peaks.

Figure 7-F. Aroclor 1254 (solid line) and chlordane (dotted line) Column 4% SE-30/6% QF-1, 200°C, carrier flow 70 ml/min.

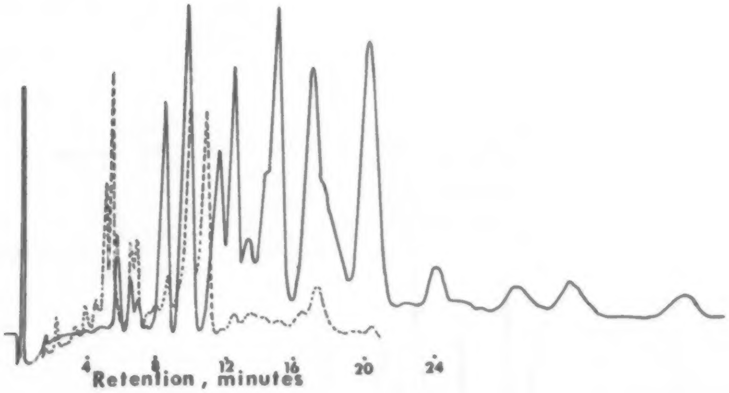
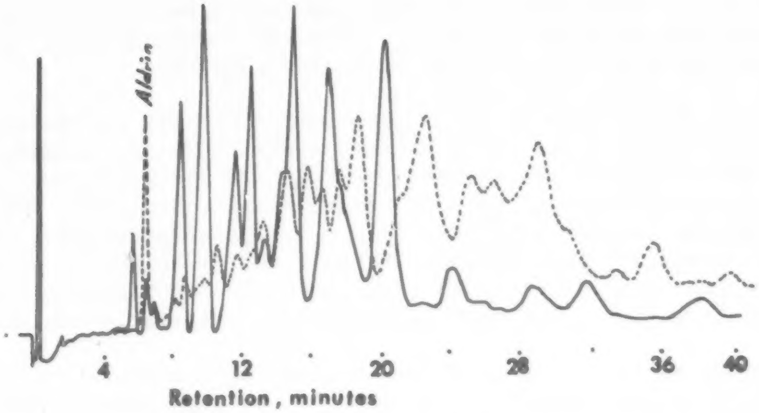


Figure 7-G shows a mixture of Aroclor 1254 with toxaphene. Analyses of toxaphene, chlordane, and PCBs are further confused because the chromatograms of environmental samples never exactly resemble those of standards. Chlordane is not very widespread in environmental samples, so its mutual analysis with PCBs is less likely to be a problem.

Figure 7-G. Aroclor 1254 (solid line) and toxaphene (dotted line) Column 4% SE-30/6% QF-1, 200°C, carrier flow 70 ml/min.



The actual effect of PCBs on quantitation of chlorinated pesticides is highly dependent on the levels involved, the pesticide of interest, and the attenuation being used. For example, if the ratio of PCBs to pesticides is 10 ppm to 3 ppm, an attenuation can be used which will give an adequate peak for DDE while DDT (for example) and PCBs will hardly be seen. If quantitation of DDT is required, however, a lower attenuation will be required (because of its lower response) to give an adequate peak size, the DDE peak will be off-scale, and PCB peaks will be more noticeable. At a ratio of 25 ppm PCB to 3 ppm pesticide, quantitation of DDT will definitely be affected, and with 100 ppm PCB to 3 ppm pesticide and attenuation to keep DDE on scale, determination of the latter would be affected.

7A,E METHODS FOR SEPARATION AND ANALYSIS OF PESTICIDES AND PCBs

a. Published Procedures and Data

The EPA PAM contains macro and micro methods for determining PCBs in human milk in Sections 9,B,(1) and 9,B,(2), respectively. In the macro method, the milk sample (4-24 grams) is extracted with acetone and hexane, PCBs are transferred to the hexane layer by adding sodium sulfate solution, and the hexane is dried by passage through a sodium sulfate column. Part of the sample is used for a lipid determination, and the rest is partitioned with acetonitrile and then fractionated on an activated Florisil column 10 cm in height. Identification and quantitation of PCBs is carried out by EC-GC and confirmation by use of different GC columns, and the electrolytic conductivity detector (Cl-mode), chemical derivatization by perchlorination, and GC-MS of pooled samples.

In the micro method, a 0.5 gram sample of milk is extracted with acetonitrile, residues are partitioned into hexane, the hexane is concentrated, and the PCBs are eluted through a 1 gram deactivated (3 percent water) Florisil column. The eluted PCBs are further separated from chlorinated pesticides on a micro silicic acid column. Neither the macro nor micro methods are capable of accurately identifying or quantitating absolute levels of PCBs, but they provide semi-quantitative results.

Filter paper, glass wool, and sodium sulfate are likely sources of PCB contamination in the macro method and these materials must be thoroughly precleaned with pesticide grade solvents as described in Section 3K. Each sample analyzed requires a total volume of ca. 2000 ml of solvent, and care must be taken in concentration of this large volume to the final 1-5 ml for analysis. One blank and one fortified goat's milk sample should be run with every set of 10 human milk samples for both the macro and micro methods. Details for preparing these samples are described in Section 9,B,(1), XIV and 9,B,(2), X of the EPA PAM. The amount of Florisil needed for a proper elution pattern should be determined for each different lot by elution of analytical standards. Proper separation of PCBs and pesticides on the silicic acid column should be checked by chromatographing standard compounds and analyzing both eluate fractions. The Aroclor standard providing a chromatogram most closely resembling that of the sample should be used for quantitation of that sample.

Analysts inexperienced with the method should be guided through the procedures at least four times by a person experienced with the procedure, using duplicate samples already analyzed by the experienced worker. Then the analyst should be required to demonstrate proficiency on an additional set of four spiked samples without aid before handling actual samples.

The EPA Manual also describes the separation of PCBs from DDT and its analogs by the method of Armour and Burke (139) (Section 9,C), and a thin layer method for semiquantitative estimation of PCBs in adipose tissue (Section 9,D). Section 9,E illustrates chromatograms of different Aroclors on 4% SE-30/6% OV-210 or QF-1 and 1.5% OV-17/1.95% QF-1 GC columns, and Section 9,F tabulates relative retention values and response values of six Aroclors on OV-17/QF-1, SE-30/QF-1, and OV-210 columns. Retention indices have been calculated for all 210 possible individual PCBs on 13 GC phases, and recommendations were made for the best phase combinations for separations (OV-210, Apiezon L, and OV-225 were among the best single columns; OV-3 + CHDMS and OV-3 or OV-25 + poly MPE were the most discriminating pair) (140). HPLC and capillary column GC have also been used to separate PCB mixtures (141, 142).

Crist and Moseman (143) of the EPA reported a simplified micro perchlorination method for determination of PCBs in biological samples. A sample was cleaned-up by the modified MOG procedure (Section 7A), and the PCBs were perchlorinated with $SbCl_5$ to decachlorobiphenyl (DCB), which was cleaned-up by hexane partitioning and chromatography on a 1.6 gram column of activated Florisil. DCB was eluted with 7 ml of hexane. The amount of DCB determined in the sample by EC-GC was converted to the desired Aroclor through multiplication by the quotient obtained from the average molecular weight of Aroclor divided by the molecular weight of DCB. Recovery of as little as 0.1 ppm of PCBs in 500 mg of tissue extract was 79-99 percent. Human milk and fortified goat milk samples were also analyzed. The presence of impurities in $SbCl_5$ reagent that can cause erratic recoveries of PCBs was noted by Trotter and Young (144), and DCB impurity was detected in various brands of the reagents used in the Crist and Moseman procedure (143).

b. PCB Cleanup and Separation Systems

Depending upon the particular pesticides and PCBs present, the amounts of each, and the purpose of the analysis, it may or may not be necessary to separate PCBs and pesticides present in the same extract before the determinative step. Some combinations may permit quantitation of each without prior separation, others will require a separation before determination, and still others may require a separation procedure that destroys or converts some of the compounds to permit quantitation of those remaining unchanged.

PCBs are eluted with 6 percent ethyl ether-petroleum ether in the modified MOG procedure described in the EPA PAM, Section 5,A,(1) and in the FDA PAM multiresidue procedures, Sections 211 and 212. They elute with eluent I of the alternative methylene chloride elution system (Section 7H of this Manual and Section 252 of the FDA PAM). A study by Lieb and Bills (145) found that the storage temperature of Florisil after initial activation (overnight, 130°C) influenced the GC pattern obtained for Aroclor 1254 separated from lipids on a column of the Florisil. To avoid selective adsorption of some PCB components and erroneous PCB analyses, storage of activated Florisil at room temperature was recommended. This, however, is in opposition to the procedure recommended for routine pesticide work (continuous storage at 130°C until use) and should be studied further. Hydroxy PCB metabolites extracted from cow's milk were cleaned up by extraction with aqueous alkali and re-extraction of the acidified aqueous solution with organic solvent prior to further TLC cleanup and GC-MS determination (146).

Polychlorinated terphenyls (PCTs) are also recovered by the multiresidue procedure described in Section 5,A,(1), but these compounds elute from the GC column much later than OCl pesticides and PCBs and therefore do not interfere. To determine PCTs, GC parameters must be altered to provide more rapid elution and greater sensitivity. The spectrometric and GC properties of 22 PCTs have been reported (147).

The method of Armour and Burke (139) has been most used for pesticide-PCB separation. The 6 percent ethyl ether-petroleum ether Florisil column eluate or eluate 1 of the alternative procedure (Section 7G of this Manual) is concentrated to an appropriate volume and a 5 ml or smaller aliquot applied to a column of partially deactivated silicic acid and Celite, standardized before use to effect the best possible separation between p,p'-DDE and Aroclor 1254. Petroleum ether followed by acetonitrile-hexane-methylene chloride (1:19:80 v/v) are used to elute the column, both fractions being collected in a K-D evaporation flask. The eluates are concentrated and subjected to EC-GC. Mixed results have been reported with this silicic acid separation system. PCBs and polychlorinated terphenyls split between the two fractions (EPA PAM, Section 9,C, Table 1) as do the pesticides aldrin and p,p'-DDE (Canadian PAM, Section 7.5). Polychlorinated naphthalenes (148) and dioxins elute in the first fraction and most other chlorinated pesticides (e.g., chlordane, toxaphene, DDT, heptachlor, lindane) in the second. Because of the division of some compounds between the two eluates, GC columns must be carefully chosen to separate the components present in each fraction. The tables of relative peak heights and peak retentions in the EPA PAM can help in this selection. The chemist running this procedure for the first time should perform a sufficient number of recovery trials with spiked samples to gain confidence in its reliability. Impurities present in silicic acid adsorbent batches, their effect on separations, and means for their removal have been described (149). Pesticide-PCB separations were found reproducible only for individual batches of adsorbent.

A slightly modified version of the Armour-Burke method is detailed in the Canadian PAM Section 7.5, and the method has been miniaturized for determining chlorinated pesticide and PCB residues in fish. In the latter method, the sample is dried with Na_2SO_4 and packed into a column, which is eluted with petroleum ether. Cleanup and separation of the extract is on 1 cm (id) Florisil and silica gel columns (150).

Other columns used in various multiresidue cleanup procedures provide at least partial separations of organochlorine pesticides from PCBs. These include columns of activated alumina (23; Section 7M), silica plus alumina (21; Section 7M), and charcoal (151). Section 251.2 of the FDA PAM describes derivatization and micro-column chromatography procedures for removal of DDT and related compounds from extracts containing PCBs. Cleaned-up extracts are treated with alkali to convert

DDT to DDE and TDE to its olefin; PCBs are unchanged. Subsequent oxidation of the solution with chromium trioxide in acetic acid converts DDE and TDE olefin to dichlorobenzophenone, but again PCBs remain intact. PCBs are then separated from polar dichlorobenzophenone by elution with petroleum ether from a micro activated Florisil column. Dichlorobenzophenone is eluted, if required, with ethyl ether-petroleum ether (1:1 v/v). Recoveries of Aroclors 1242, 1254, and 1260 ranged from 77-100 percent (0.4-56 µg amounts), while DDT, DDE, and TDE were recovered (as dichlorobenzophenone) between 5-86 percent (2-33 µg). The same reactions used in this GC determinative procedure have been applied to TLC estimation (Subsection d.) and confirmation (Subsection e.) of PCBs. Other techniques for separating PCBs from DDT and its analogs by chemical derivatization and column chromatography include: dehydrochlorination with 1,5-diazobicyclo(5.4.0)undec-5-ene reagent (152); sodium dichromate in acetic acid plus sulfuric acid for conversion of DDE to dichlorobenzophenone without affecting DDT, TDE, or PCBs (153); a silica gel tube with MgO catalyst for conversion of DDT to DDE and TDE to DMU without effect on PCBs (154); and heating cleaned-up fish or serum extracts with KOH/ethanol to convert OCl pesticides to alkenes, oxidation with Cr_2O_3 to more polar compounds, and separation from PCBs on a Florisil column (155).

Aroclor 1254 residues in blood have been determined by extraction with hexane-saturated acetonitrile and cleanup on an alumina column. Eluates were analyzed by EC-GC on an OV-1 column (156). PCT, PCB, and DDT residues in blood (5-11 ppb) were determined by heating with ethanol and KOH to dehydrochlorinate DDT, extracting with hexane, washing with H_2SO_4, and chromatographing on a mixed silica + Florisil + Na_2SO_4 column. The hexane eluate was concentrated and analyzed by EC-GC on a 2% OV-1 column, and confirmation was by MS (157).

c. GC Quantitation of PCBs

One of the most difficult aspects of PCB quantitation is to obtain a match between the sample and a standard. Environmental samples seldom have a GC peak pattern that will exactly match any Aroclor standard, and even commercial preparations of PCBs vary in abundance of minor components from batch to batch. In addition, detector response to different PCB isomers can vary by as much as 10,000-fold, so that accurate quantitation is impossible. The most widely used practical approach is to compare the total area or height of detector response for the residue peaks to the total area or height of response obtained under the same conditions for a known weight of the commercial Aroclor standard with the most similar pattern. Only those peaks from the sample that can be attributed to chlorobiphenyls are used, and these peaks must also be present in the chromatogram of reference materials. If the presence of more than one Aroclor is clearly indicated, the residue may be quantitated using mixtures of Aroclor standards judged appropriate for different portions of the sample chromatogram.

The procedure of Webb and McCall (158) has an advantage compared to this method in that residues can be quantitatively measured on a GC peak-to-peak basis against a series of reference Aroclors with known weight percentage compositions for individual peaks. Reference Aroclors 1016, 1242, 1248, 1254, and 1260 have been characterized using a Hall electrolytic conductivity detector for Cl measurement and chemical ionization MS with single ion monitoring for molecular weights (159). A ten laboratory collaborative study of PCB quantitation in synthetic standard mixtures, milk, and chicken fat samples indicated that greater overall precision was possible using the individual peak method compared to total area or height (160).

Other quantitation approaches that have been attempted include perchlorination of all PCB compounds with $SbCl_3$ to a single derivative (decachlorobiphenyl) (143, 161; Subsection 7A,E.a.), estimation of the weight of PCB injected by dividing the retention time x peak height for all PCB peaks by the product of peak height and retention time for 1 ng p,p'-DDE on the same GC column (162), and peak resolution and matching by a computer (163). GC-MS with individual mass monitoring using a minicomputer-controlled spectrometer has been reported (164). This method provided sensitive qualitative and quantitative analysis of sediment extract without the need for elaborate column adsorption separations prior to GC. Beroza and Bowman's p-values have been applied to quantitation of p,p'-DDT in the presence of non-resolved PCB peaks and results within 11 percent of actual were reported (165). The USFDA approach to chemical profiling of PCB content in a sample to select the most suitable quantitation standard has been discussed.

d. PCB Estimation by TLC

The semiquantitative TLC procedure (167) for determination of PCBs in adipose tissue utilizes the 6 percent eluate of the Florisil cleanup. The concentrate is treated with KOH to dehydrochlorinate DDT and DDD to their olefins, thereby eliminating the problem of separating the pesticides from the PCBs. Any interfering DDE is then oxidized to *p,p'*-dichlorobenzophenone which has an R_F value different from the PCBs on a silver nitrate-impregnated alumina layer developed with 5 percent benzene in hexane. The PCBs give one spot for all formulations, and this is quantitated against a standard Aroclor 1254 or 1260 spot run on the same plate. The best standard can be chosen after examining a preliminary GC trace. The final values obtained by this method should be considered as approximations, with a precision of roughly ±50 percent indicated by recovery studies. The minimum level of detection is ca 1 ppm by exposure of the layer to UV light. An EPA human monitoring program for PCB levels in adipose tissue has utilized this TLC procedure with confirmation by combined GC-MS (168).

e. Confirmation of PCBs

Confirmation of PCBs has been obtained by perchlorination (143, 161) and alkali treatment (169). The stability of PCBs to alkali makes the latter reaction useful for confirming the identity of PCB residues, and at the same time, conversion of DDT to DDE by the alkali removes some interference to quantitation of PCBs. Treatment with alkali also provides additional cleanup for many sample types. Resistance to oxidation with chromic acid-acetic acid reagent is also useful evidence for identifying PCBs in the presence of reactive pesticides such as DDE and DDT (167) and chlorinated naphthalenes (170).

Two-dimensional (171) or multi-development reversed phase (172) TLC systems, which separate PCBs from DDE, DDT, and other pesticides, can aid identification. PCBs are destroyed by UV irradiation, but many pesticides may be altered as well. Toxaphene survives UV treatment that destroys PCBs and can be confirmed in mixtures in this way. Mirex, a late eluting pesticide which usually is not interfered with by PCBs, also withstands UV irradiation and can thus be confirmed. Irradiation with controlled UV wavelengths has provided identification and determination of aldrin, dieldrin, heptachlor, and heptachlor epoxide in mixture with PCBs. Photoisomerization reactions of the pesticides, producing products with longer retention times, were induced with wavelengths $>$290 nm; subsequent irradiation with wavelengths $>$230 nm yielded photodechlorinated products of PCBs with shorter retention times (173). Most organochlorine pesticides are destroyed by reaction with HNO_3-H_2SO_4 whereas PCBs and toxaphene are unaffected. Chlorinated pesticides were selectively detected in the presence of PCBs by use of a modified Coulson conductivity detector at 600°C with a hydrogen flow of 1-2 ml/minute (174). Mirex and PCBs have been differentiated based on the low sensitivity of the Hall detector for the latter (175). A collection of spectra helpful in confirming isolated residues of PCBs has been published.

7A,F DETERMINATION OF POLYBROMINATED BIPHENYLS

Polybrominated biphenyls (PBBs) were manufactured for use as a fire retardant from 1970 to 1973. Since the summer of 1973, when PBBs were accidentally mixed with dairy feed resulting in the contamination of livestock and food products, the sensitive determination of low levels of PBB residues has been of interest to the FDA and the EPA. One commercial PBB fire retardant (Firemaster BP-6) has been chemically and toxicologically evaluated; 13 different polybromobiphenyls were found plus a brominated naphthalene contaminant, and biological effects were ascribed to PBBs (177).

A rapid method has been developed for analysis of plasma, feces, milk, and bile using all disposable glassware to reduce the amount of laboratory background and cross-contamination of samples (178). The authors found that this type of equipment was necessary because methods that had proven to be effective for decontamination of PCBs were not effective for PBBs. The methodology involved multiple extraction of ethanol-denatured sample (except for feces) with petroleum ether-diethyl ether (1:1 v/v) in a disposable test tube, followed by cleanup on a miniature adsorption column packed in a 23 cm disposable Pasteur pipet. The column contained Florisil, silica gel, and sodium sulfate in different proportions, depending on the sample. The column was eluted with 5 or 10 ml of petroleum ether-benzene (98:2 v/v) into a disposable screw top vial. Determination of PBBs in the concentrated eluate was made by EC-GC on a silanized 5% OV-17 column. Mean recoveries for the six major components of a commercial PBB mixture were approximately 96 percent for plasma, 59 percent for feces, 98 percent for milk, and 89 percent for bile at 0.05-50.0 ppm levels. The maximum background level was 0.0007 ppm for the major hexabromobiphenyl peak, corresponding to a minimum detectable limit of ca. 0.001 ppm.

The separation and characterization of PBBs by chromatography and spectroscopy have been studied (179). Columns containing 1% SE-30 or 2% OV-17 were used for GC-FID-MS, 5 µm silica gel 60 (Merck) columns for HPLC (UV detection), and paraffin-coated kieselguhr for reversed phase TLC. In addition to quadrupole MS, NMR and UV spectroscopy were evaluated.

PBB residues in dairy products were determined at 7 ppb levels by co-extraction from the sample along with fat, separation by GPC, EC-GC on an OV-101 column, and confirmation by TLC (180).

7A,G SEPARATION AND DETERMINATION OF DIOXINS

Laboratories that have conducted environmental monitoring projects for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) have developed and applied analytical cleanup systems and mass spectrometric methods of analysis for sub-ppt to 100 ppt levels of TCDD residues in environmental, biological, and human samples and chemical formulations (181-192).

The cleanup methods involve acid, base, and/or neutral extraction procedures followed by column chromatography using Celite, alumina, and silica gel columns. The mass spectrometric analysis methods include direct probe high resolution single and double ion monitoring, low resolution (packed column) GC/low mass resolution MS analyses, low resolution GC/high mass resolution double ion monitoring, high resolution (capillary) GC/low mass resolution analyses and high resolution (capillary) GC/high mass resolution multiple (three ion) monitoring MS techniques.

The preparation and characterization by GC-MS of a series of dioxin standards useful for residue quantitation have been described (91).

7A,H DETERMINATION OF ETHYLENETHIOUREA (ETU)

Because of its toxicological significance, constant occurrence as a terminal residue following crop treatments with ethylenebisdithiocarbamate fungicides, and its actual presence in technical ethylenebisdithiocarbamates, analytical methods for determination of ETU are becoming increasingly important. A method for ETU in apples (193) was based on reaction with benzyl chloride to give 2-benzylmercaptoimidazoline, which is subsequently treated with trifluoroacetic anhydride to yield 2-benzylmercapto-N-trifluoroacetylimidazoline. This derivative is measured by EC-GC.

ETU residues were measured in various crops by methanol extraction, alumina column cleanup, and derivatization with 1-bromobutane in the presence of DMF and sodium borohydride. The resulting 2-butylmercaptoimidazoline was measured down to 0.01 mg/kg with an FPD detector (194). A similar method that determines ETU in milk, fruits, and vegetables as the same derivative has been collaboratively studied, and tentative evaluation indicates successful results (195).

EC-GC as well as S-mode FPD-GC has been used to determine ETU residues from crops after derivatization with m-trifluoromethylbenzyl chloride (196). The trifluoroacetylated S-benzyl derivative has also been used to determine ETU residues on tomatoes (197). ETU residues on fruit and vegetable crops were determined at 0.01-0.1 ppm levels without derivatization (198). After methanol extraction and cleanup by hexane/aqueous NH_4Cl partition and alumina column chromatography, GC was performed on a 3% Versamid 900 column with S-mode FPD detection. Recoveries ranged from 62-95 percent.

The occurrence, chemistry, and metabolism of ETU and analytical methods for its determination have been reviewed (199).

7A, I DETERMINATION OF CONJUGATED PESTICIDE RESIDUES

Pesticides and pesticide metabolites are known to form carbohydrate (glycoside, glucuronide), amino acid, sulfate, alkyl, and acyl conjugates in various plant, animal, and soil systems. Because of the potential biological activity of many of these conjugates, their identification and determination has become an important task for the pesticide analyst.

Because conjugates are, in general, more polar and less lipophilic than the parent pesticides, analytical methods are designed to take into account these differences. In addition, the lability of certain conjugates may dictate the analytical approach taken when isolating and identifying the intact compound or a derivative, e.g., the need for protection of labile moieties from hydrolysis during extraction or the choice of column LC rather than GC for separation of thermally unstable or nonvolatile conjugates. Analysis of enzymatic or chemical hydrolysis products is useful confirmatory information for conjugates that have been identified intact or may serve for the quantitation of a conjugate residue.

Different types of mass spectrometry (Section 8L), including electron impact, chemical ionization, field desorption, and laser ionization, are probably the most powerful and widely used tools for structural analysis of conjugates. The field desorption method is especially useful due to its applicability to polar materials. NMR, particularly using proton nuclei with the sensitive Fourier transform technique, is another important aid for structure elucidation (Section 8K), as are traditional IR and UV absorption spectrometry and micro-IR (Section 8J).

Specific isolation methods depend on the exact nature of the conjugate of interest and the sample matrix. Most conjugates are extractable with water, alcohol, and water-alcohol mixtures from insects, plants, or tissues. Samples may be freeze-dried and pre-extracted with an organic solvent to remove lipophilic materials. Purification, separation, and concentration of conjugates have been carried out using simple solvent partitioning, counter-current liquid-liquid distribution, extraction with liquid anion-exchangers, Amberlite XAD-2 polymer columns, silicic acid columns, Porapak Q resin columns, Sephadex LH-20 gel columns, DEAE-cellulose and DEAE-Sephadex anion-exchange columns, Sephadex G gel columns, Biogel P columns, cation-exchange resin amino-acid analyzer columns, liquid anion-exchange paper chromatography, TLC, and GC of conjugates either directly or after forming a volatile derivative.

Most analytical work on pesticide conjugates to date has been conducted for structural identifications or metabolism studies. The usual radiotracer detection techniques are widely used in metabolism research. A review of analytical methods for different conjugate types, including many literature references, and examples of applications to different research problems will be found in reference (200). This volume also contains information on the nature and analysis of "bound" or unextractable pesticide residues. One approach to the analysis of bound residues was reported for chloroaniline bound to lignin fractions of plants based on release by pyrolysis (201); pyrolysates containing intact chloroanilines were collected and derivatized as trifluoroacetanilides, which were purified and determined by EC-GC.

Relatively little attention has been given to the recovery of pesticide conjugates by analytical procedures designed to determine the parent residues. When the problem is addressed, the usual approach is that which was taken to determine carbofuran residues in plant and animal samples (202). The major carbofuran metabolite produced in animals is 3-hydroxy-carbofuran, present as the water soluble glucuronide conjugate. The analytical procedure for intact carbofuran residues was modified to include a mild acid hydrolysis, the purpose of which was to free the conjugated forms of the pesticide metabolite and allow an organic solvent extraction that included the unchanged parent compound.

A few similar procedures for other pesticides have been published, the hydrolysis step in some cases serving both to break the conjugate and to hydrolyze the parent pesticide to a new form prior to determination (e.g., hydrolysis of carbamate insecticides to the corresponding phenol, which is derivatized, cleaned-up, and determined by GC). Conjugates of 2,4,5-T in biological samples have been broken and the free acids released by a basic hydrolysis step (203). Residues of conjugated iodofenphos phenol metabolites were recovered from liver and kidney tissue by extraction with ethanol-water-1N sodium hydroxide (90:10:1 v/v), hydrolysis with 1N sulfuric acid, and hexane + ethyl ether extraction (204).

7A,J REVIEWS OF ANALYTICAL METHODS FOR PESTICIDES, PCBs, AND OTHER NON-PESTICIDE POLLUTANTS

The analysis of residues of various classes of pesticides has been reviewed in special issues of the Journal of Chromatographic Science edited by M. C. Bowman (205). Organophosphorus insecticides and metabolites, carbamate insecticides and metabolites, herbicides and metabolites, insecticide synergists, chlorinated insecticides and congeners, and fumigant residues have been covered. A review of the determination of fungicide residues (206, 207) and more recent coverage of herbicide residue determinations (89) have been published.

Published reviews have covered the extraction, cleanup, and chromatographic detection and quantitation of PCBs and other non-pesticide environmental pollutants such as chlorinated naphthalenes, dibenzofurans, and dibenzodioxins (208, 209); USDA studies on the sources, analysis, and environmental monitoring of dibenzodioxins and dibenzofurans (210); the utilization of GC coupled with chemical ionization and electron impact MS for analysis of these compounds and pesticides (211); the chromatography of PCBs (212); and PCB interference and analysis in terms of the AOAC-FDA multiresidue method for chlorinated pesticides (213).

7A,K REFERENCES

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Section 8

CONFIRMATORY PROCEDURES

8A REQUIREMENTS FOR POSITIVE CONFIRMATION OF PESTICIDE IDENTITY

Obtaining convincing identification of a trace residue is a major task of the pesticide analyst. The identity of pesticide residues should always be confirmed by a method different from that used in the initial determination since interpretation of results (e.g., decisions of a legal or health nature) as well as reliable quantitation (selection of standards) depend on correct identification. Multiresidue GC analytical methods do not provide irrefutable identification since interfering materials and artifacts are often observed, and metabolic and decomposition products may be encountered.

A specific example of a serious identification problem is the determination of the PCBs which are easily mistaken for pesticide residues such as *p,p*'-DDE and *p,p*'-DDT. Another important example concerns overlapping peaks when foods are screened for tolerance levels: a 4% SE-30/6% QF-1 column may give peaks at essentially identical retention times for endrin and *o,p*'-DDT, for Endosulfan I and *p,p*'-DDE, and for β-BHC and lindane. Both DDT and DDE are very common pesticides with rather high tolerance levels. Thus, if the analyst is unaware that endrin and endosulfan may produce corresponding GC responses, he may conclude that observed peaks indicate only insignificant quantities of DDT and DDE relative to tolerance levels and that no further work is necessary. Unfortunately, what appears to be insignificant response for DDT and DDE is very substantial response for endrin and Endosulfan I because of lower GC sensitivity to these compounds and lower tolerance levels; therefore, confirmation of identity is mandatory (1).

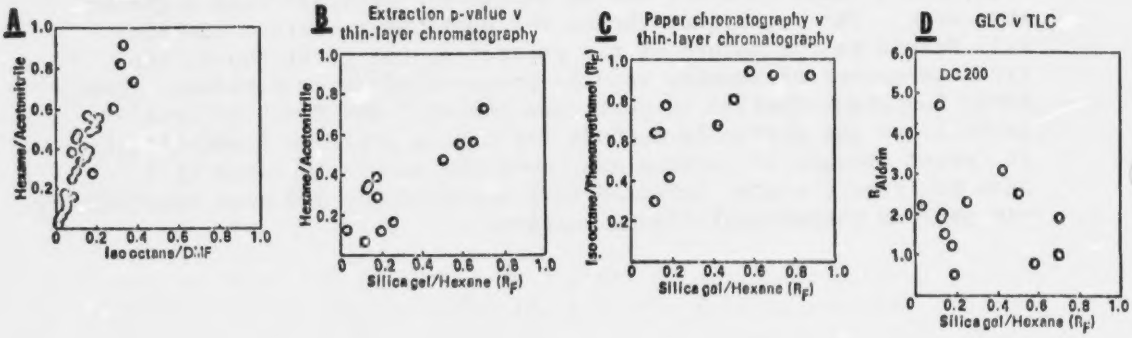
Confirmatory evidence is especially important with the relatively nonspecific EC detector. One difficulty is that determinations of very low pesticide concentrations are usually required, and many potentially useful confirmatory methods (e.g., infrared spectroscopy) require a greater quantity and/or purity of pesticide than might be available. The techniques chosen for confirming various residues will depend on the nature of the pesticide, the level found, the type and amount of sample, and the presence of other residues. The lower the concentration of pesticide present, the fewer or less certain are the available methods for making positive identification. If larger amounts of residue are found and can be isolated in a reasonably pure state, infrared (IR) spectroscopy and mass spectrometry can provide unequivocal identification.

Considerations of set theory (2) indicate that three independent "equivocal" results are required in order to be confident of the identity of a pesticide residue. These might be elution in a certain fraction from a liquid chromatography cleanup column, a GC retention time, and a positive response of a selective GC detector. Another possible combination that would be a basis for confidence is the GC retention times from a polar column and a nonpolar column plus a R_F value from paper chromatography (PC) or thin layer chromatography (TLC) or an extraction p-value. Still another would be a GC retention time, a PC or TLC R_F value, and the GC retention time of a derivative formed by a chemical or photochemical reaction.

The dependence or independence of measured values was studied by Elgar (3) who reported that many widely used confirmatory methods may not give truly independent evidence of identity since they are measuring the same chemical or physical properties. Thus, care must be exercised when deciding which methods to use in combination to avoid doing a great deal of work without gaining additional useful information. Examples of highly correlated (not independent) values include GC retention times on certain stationary phases (Figure 5-N in Section 5); PC or TLC R_F values from certain adsorbent/solvent systems; p-values in different solvent pairs; and PC, TLC, and p-values. These combinations will not provide independent information for confirming residue identity.

In Figure 8-A, the correspondence between extraction p-values in hexane/acetonitrile and isooctane/DMF solvent pairs (A), and p-values in hexane/acetonitrile and TLC R_F values with the system silica gel/hexane (B) is shown by the generally straight line along which the plotted data points lie. The independence of TLC and PC data (Figure 8-A, (C)) and GC and TLC data (D) is illustrated by the scatter of the points. Clearly, many combinations of alternative columns, selective detectors, p-values or PC or TLC, and chemical derivatization can be applied for purposes of confirmation.

Figure 8-A. Degree of correspondence between different types of data for residue confirmation. A = extraction p-values, B = TLC vs p-values, C = PC vs TLC, D = TLC vs GC



When the analyst is making pesticide identifications, common sense is necessary. An example of misapplied common sense would be reporting methyl or ethyl parathion in human fat; metabolically it is virtually impossible for parathion to persist per se and to appear in a tissue or body fluid (except gastrointestinal). The persistence of heptachlor would also be very unlikely because body metabolism normally converts it to heptachlor epoxide. Chromatography with EC detection of human adipose tissue from the general population often produces peaks with retention characteristics very close or identical to the RRT_A values for α-BHC and/or $\underline{o},\underline{p}'$-DDE. However, the presence of these compounds has rarely, if ever, been confirmed. In these instances, the peaks in question represent artifacts that happen to have the same retention times as these pesticides, and careful confirmation by ancillary techniques would provide the proper identification.

In summary, since all methods and tests regularly used in residue analysis are presumptive in nature (the behavior of an unknown is compared to that of a known, standard material), it is most desirable to use a number of tests that measure different chemical or physical properties. The initial GC method should have been proved to recover and detect the pesticide residues of interest, and it is desirable that data are available on the behavior of many pesticides and their metabolites and degradation products in the various operations that comprise the method. The analyst should be familiar with and capable of fully using and interpreting this data and all other available information, including pesticide usage, the chemistry and metabolism of residues, common artifacts from sample substrates and reagents, and the possibility of interfering residues, such as PCBs and phthalate esters. Analytical conclusions must be reached with an open mind, common sense, and reasonable judgment. The extent of confirmatory effort and the exact procedure chosen will depend on factors such as the history and significance of the sample; nature and level of the residues; sample type; purpose of the analysis; and practical considerations such as time, cost, number of samples, and available instrumentation. Alternatives to confirmation of residues in all samples are discussed in Section 1E. "Unusual" residues should be verified in all analyses, even at low levels, to support a decision to devote further effort to tracing their origins. Confirmatory methods should yield identical results with both the suspected sample residue and standard reference material subjected concurrently to the same tests. Similar concentrations of the sample and standard should be used in the comparative testing to demonstrate quantitative as well as qualitative confirmatory evidence (FDA PAM, Section 601). The following subsections discuss the more widely used confirmatory procedures.

8B GC RELATIVE RETENTION TIMES

In most laboratories, the initial, tentative identification of a pesticide residue results from a multiresidue procedure involving extraction, cleanup, and gas chromatography. Tables of GC retention times for particular column-detector combinations are normally used for the tentative identification. The recovery of a residue through the preliminary cleanup steps should not be overlooked as valuable, supplemental confirmatory evidence. This is particularly true when such characteristic properties as the ability to withstand acid or alkali treatment or elution in a particular fraction from an adsorbent column is involved.

The following guidelines are useful for the proper utilization of retention times in making compound identifications.

a. The use of relative retention or Kovats' retention indicies (4) rather than absolute retention is more reliable (Subsection 5N in Section 5).

b. Be highly suspicious of any peak with a calculated relative retention value (RRT) that does not precisely match that of your standard or that of the tables (EPA PAM, Section 4,A, (6)). A simple aid is to co-chromatograph some pure standard of the suspect compound along with your sample extract and observe the peak configuration compared to that of the sample alone. If some distortion is evident in the configuration of a given suspect peak, the identification can be safely negated.

c. If cleanup is used on the sample, always run the elution fractions separately. Do not pool the elution cuts. Selective adsorption combined with GC retention characteristics provides a valuable identification tool for pesticide analysis.

d. NEVER rely on one GC column for positive identification. Use an alternative column providing a completely different peak elution pattern.

As illustrated in Figure 5-N in Section 5, the combination of a nonpolar DC-200 column with a slightly polar DC-200/QF-1 column (plot A) is not very useful for confirmation. Another highly correlated pair of phases is slightly polar 4% SE-30/6% QF-1 with slightly polar 1.5% OV-17/1.95% QF-1. To the contrary, a combination of DC-200 with highly polar DEGS (plot B) or highly polar OV-210 with OV-17/QF-1 (plot C) would be a good choice. Other complimentary pairs are SE-30/QF-1 with either DEGS or OV-210.

Specific examples (5) of the utility of at least two different GC columns for sample diagnosis include the following. Identity of certain early eluting BHC isomers, particularly the alpha isomer, may be hindered by the presence of hexachlorobenzene. The latter is co-eluted with α-BHC on silicone columns and with β-BHC on Apiezon, but all three compounds are resolved on a polar cyano-silicone column. Dieldrin and p,p'-DDE are difficult to resolve on a number of single phase silicone columns but are separated on Apiezon, cyano-silicone, and trifluoropropyl silicone (QF-1, OV-210, SP-2401). On Apiezon, dieldrin elutes before DDE while the order is reversed on the cyano-silicone column. On the QF-1 or OV-210 column, dieldrin elutes far later than p,p'-DDE, to the extent of about 1.4x at 180°C column temperature.

8C SELECTIVE GC DETECTORS

The EC detector, being rather non-specific, responds to any electron capturing compounds injected in addition to pesticides. For this reason, interpretation of results from EC-GC is facilitated if additional chromatograms are run using one or more of the highly selective detectors. The microcoulometric (MC), flame photometric, or conductivity detectors, described in Subsections 5D, 5F, and 5G, are especially useful for confirmation. Because interference peaks may occur with even the most selective detectors available, the absence of a peak is really more conclusive than a positive response. For example, if a peak on an electron capture chromatogram suspected of being a chlorinated pesticide does not appear when the sample is injected into a chromatograph with the same column and a MC or Coulson conductivity detector in the Cl mode, this is convincing evidence that the original peak was definitely not due to a chlorinated pesticide but most likely an artifact with a coincident retention time. Appearance of the peak in the MC or conductivity chromatogram indicates that the peak was due to a halogenated compound, but further confirmation is still required to prove that the peak truly represents the pesticide of interest and not an artifact.

Because of the selectivity of its filters, the flame photometric detector (FPD) greatly simplifies confirmation of sulfur- and/or phosphorus-containing residues. Identification of a thiophosphate is unequivocal if (1) its retention ratio on a given column matches with a standard, (2) the compound elutes in the correct fraction from a cleanup column, (3) it is detected by the FPD detector, and (4) the sulfur (394 nm) to phosphorus (526 nm) response ratio of the FPD matches the standard (Subsection 5F).

Use of appropriate specific titration cells in the MC detection system (Subsection 5D) provides additional evidence of identity for compounds containing halogen, sulfur, or nitrogen. The detector has relatively low sensitivity, therefore requiring a greater concentration of the extract, larger sample injection, and/or a larger initial sample than for the EC detector.

The selectivity of an EC detector can be improved if the products formed in an EC detector are allowed to pass to a second column with another EC detector; the resultant distinctive peak pattern can provide identification of OCl pesticides and PCBs (6).

8D THIN LAYER CHROMATOGRAPHY (TLC) R_F VALUES

Experimental aspects of TLC and its use for screening and quantitation of residues were covered earlier in Subsections 6Q through 6T in Section 6.

TLC is perhaps the simplest confirmation technique for GC when levels of residues present are high enough. An aliquot of cleaned-up extract is evaporated to near dryness, a suitable solvent is added, and a detectable quantity of the sample is spotted on a thin layer plate together with appropriate standards. An agreement of about ± 2 mm in migration distance of the sample and standard spots is considered adequate since the movement of the sample is likely to be affected by co-extractives despite cleanup steps. If the sample contains several pesticides, different solvents and/or adsorbents may be required before all are separated and matched with standards. Mixing together the sample and a standard and observing whether separation occurs (co-chromatography) is another procedure for making comparisons.

It is best not to rely on published or previously determined R_F values for confirmations because differences in development conditions from run to run cause these values to be non-reproducible. Standards and samples should always be run on adjacent areas of the same plate if possible. If R_F values must be used, the value relative to the R_F of a standard compound X run on the same plate (R_X value) will be more reliable than the absolute R_F value for many of the same reasons that relative GC retention times are more reliable than absolute retentions. Chlorinated pesticides are often referred to DDD, and phosphates to parathion, in calculating R_X values.

Although TLC is very widely applied for pesticide confirmation, results may not always be conclusive. TLC confirmation of many pesticides, such as toxaphene and chlordane, is greatly influenced by the degree of cleanup on the sample extract and the level of detection. Oils and waxes will particularly interfere with TLC, causing streaked zones and/or distorted R_F values which may completely negate its value for confirmation. The 15 percent ethyl ether-petroleum ether Florisil column extract normally requires further cleanup prior to TLC (FDA Pesticide Analytical Manual, Section 411.5).

Detection reagents yielding spots of different colors with different pesticides are especially valuable for confirmation. Diphenylamine-zinc chloride reagent provides such differentiation for chlorinated pesticides, various shades of purple, grey, green, and reddish-orange colors are produced on the layer after spraying and oven heating (FDA PAM, Section 612). Identification of naturally fluorescent pesticides is aided by heating the chromatogram, causing specific alterations in recorded spectra (7). This heating procedure may, however, increase background fluorescence from co-extracted compounds also present in the sample. TLC after fluorogenic labeling (8) of pesticide residues is a combination of chromatography with chemical derivatization (Subsection 8G) which can provide very specific detection of certain residues. If sufficient pesticide is present in the thin layer spot, scraping, collecting the adsorbent, and eluting the compound followed by mass spectrometry (Subsection 8L) can provide unequivocal identification.

It was mentioned earlier in this section that if additional independent information is to be gained by running PC plus TLC or TLC in more than one system, the systems must be very carefully chosen to be truly "different". The use of multiple R_F values for identification purposes was studied by Connors (9), who found that useful, uncorrelated data can be obtained in several ways, such as by pairing aqueous with nonaqueous systems, acidic with basic solvents or supports, aprotic with protic solvents, polar with nonpolar solvents, hydrogen-bond donors with hydrogen-bond acceptors, or reversed phase with normal phase systems. The specific approach which might be successful depends on the chemical nature of the pesticides to be confirmed. The important point is that different thin layer and/or PC systems chosen at random will not necessarily provide the analyst with any additional, independent evidence of identity.

Permanent records of TLC plates for documentation should be made by one or more of the following methods: Xeroxing the original plate, spraying with plastic to preserve the plate, hand tracing or charting, densitometry, or color photography (10). Where available, the latter appears to be the preferred procedure.

8E HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

See Subsections 6N through 6S in Section 6 for a discussion of this topic. HPLC has been used mainly for quantitation of residues in situations where GC is either not applicable or not convenient to use. An HPLC retention time can serve as evidence to confirm GC in the same way as a PC or TLC R_F value. The liquid chromatographic system should be carefully chosen to be "different" from the GC system (i.e., adsorption rather than partition), and the independence of the data must be clearly established if it is desired to use both PC or TLC and HPLC data for confirmation. The variable wavelength UV detector allows determination of the wavelength of maximum absorption for each pesticide. Detection of HPLC effluents with a Cl-selective Coulson electrolytic conductivity detector (11) can also provide useful confirmatory evidence.

8F EXTRACTION *p*-VALUES

Extraction *p*-values (12-16) are a tool for identifying pesticides at the low ng level. The *p*-value is determined by equilibration of a solute between volumes of two immiscible liquid phases followed by the analysis of one of the phases for the solute. The *p*-value, defined as the fraction of total solute partitioning into the upper phase, can be derived from a single distribution between the solvents or from a multiple distribution, as in counter-current distribution. *p*-Values for most pesticides are appreciably different from those of normal co-extracted contaminants. The determination of these values is simplified since only relative, rather than absolute, data are required, and sensitivity is at or only slightly above the level of EC-GC.

Details including experimental procedures, formulas for calculating *p*-values and the fractional amount extracted after repeated extractions, graphs for determining specificity in a given system, and *p*-values for 131 pesticides in six binary solvent systems (hexane-90% DMSO, heptane-90% ethanol, isooctane-80% acetone, hexane-acetonitrile, isooctane-DMF, and isooctane-85% DMF) are given in Section 621 of the FDA PAM, reference (13), and Section 12.C of the EPA PAM (data for 88 pesticides in the latter). A device and method for determining *p*-values with unequilibrated solvents or unequal phase volumes are given in the FDA PAM, Section 622.1 and reference (16).

As mentioned earlier, the general technique of determining *p*-values has much in common with the use of several GC columns, PC, and TLC in identification studies since all systems may share the same partition mechanisms. Unless the analyst assures himself that the data are not correlated, it is best to use either a PC or TLC R_F value or an extraction *p*-value as one independent criterion of identity. The great advantage of *p*-values over PC or TLC is that the method is useful at levels amenable to quantitative analysis by EC-GC where sufficient residue might not be available for either of the former techniques.

8G DERIVATIZATION (CHEMICAL REACTION) TECHNIQUES

Derivatives of pesticides are prepared for various reasons, such as to decrease volatility or increase detectability for TLC; to increase volatility, stability, and/or detectability and avoid tailing peaks for gas chromatography; and to alter the structure to aid characterization. It is this latter topic that will be discussed in this subsection.

Comparison of retention times on a given GC stationary phase before and after chemical derivatization is a relatively recent innovation which is becoming increasingly important for corroboration of residue identity. Desirable characteristics of any chemical derivatization technique include:

a. A specific product should be formed with at least as much or more response to electron capture, or to other detection, compared to the parent pesticide.

b. The product should have a different retention time than the parent, preferably greater to differentiate it clearly from the background.

c. Reactions should be essentially quantitative, they should use highly pure reagents and solvents, and they should be facile and rapid.

d. A cleanup method should be available to remove any background interferences introduced by the reaction.

e. If product structures and reaction mechanisms and limitations are known, misidentifications can be avoided because the analyst can elucidate the extent and probable sources of error in the procedure.

f. Sensitivity should be at least in the 0.01 to 0.1 ppm range in terms of the parent pesticide, which is lower than the established tolerance values for most pesticides.

g. The same reaction should occur, and to the same degree, in both the sample extract and in a solution of the reference material of the suspected compound at the same concentration.

Derivatization reactions are carried out in solution, on the surface of a solid matrix, or in a GC precolumn. Reactions in solution on a microscale are most common for residue level work. The reaction usually involves heating of the reactants in a small sealed tube, after which the derivative is dissolved in a suitable solvent. If direct injection into a gas chromatograph is not possible, cleanup by solvent partitioning and/or column chromatography and concentration steps may be applied. Solid matrix reactions are generally carried out by introduction of dissolved compound onto a microcolumn composed of solid support (e.g., alumina) mixed with reagent(s). After a specified reaction time, solvent is added to elute the derivative for GC determination. The advantages of this approach are simplicity, reduced glassware needs, and ability to react many samples simultaneously. However, the same derivative as formed in a solution reaction is not always produced and/or eluted from the column in a solid matrix reaction with the same active reagent. GC precolumns are usually composed of a reagent-solid support mixture located in a heated area ahead of the analytical column. The sample is injected into the precolumn, and the derivative is formed and swept by the carrier gas onto the analytical column for determination. Speed of operation is the greatest advantage for those reactions that are rapid enough to be feasible by the precolumn technique. The chromatograph is best fitted with a special injection apparatus so injection can be made into the precolumn for derivatization or directly into the analytical column for normal operation.

The following subsections review some procedures for confirming residue identity by chemical derivatization. Table 651-A of the FDA PAM contains an extensive further listing of derivatization methods for more than 100 pesticides and related compounds of many chemical types, including comments on the level of applicability, yields, and 60 references to the original papers. A review paper on chemical derivatization in GC contains a section on pesticides (17).

a. ORGANOCHLORINE PESTICIDES

Most of the effort to date in the development of confirmatory derivatization tests has been confined to the organochlorine insecticides. For these compounds, addition, oxidation, epoxidation, rearrangement, dechlorination, hydrolysis, reduction, and dehydrochlorination are the most commonly used reactions. Examples of specific tests are shown in Table 8-1, as reviewed by Cochrane and Chau (18). A later review (19) contains more recent references to the reactions shown in Table 8-2. Table 8-3 lists selected references for OCl pesticide derivatization methods published since 1975. Still another review of reactions for chlorinated pesticides is found in reference (3). Section 7A,E,e discusses confirmation reactions suitable for PCB-pesticide mixtures. It must be realized that these reactions destroy some pesticides (and artifacts) in addition to forming pesticide derivatives.

Table 8-1

CONFIRMATORY DERIVATIZATION TESTS FOR PESTICIDE AND METABOLITE RESIDUES (18)

Pesticide	Reaction
DDT	a) Dehydrochlorination b) Dechlorination (of *p,p*'-isomer)
DDE	Oxidation
DDD	Dehydrochlorination
Methoxychlor	Dehydrochlorination
Aldrin	a) Addition: Cl_2 Br_2 *tert*-BuOCl b) Epoxidation
Dieldrin	Epoxide: cleavage rearrangement acetylation
Endrin	a) Epoxide rearrangement b) Dechlorination
Endosulfan	Sulfite reduction
Heptachlor	a) Allylic: acetylation hydroxylation dechlorination b) Addition c) Epoxidation
Heptachlor epoxide	Epoxide rearrangement
cis- and *trans*-Chlordane	Dehydrochlorination
Nonachlor	a) Dechlorination b) Dehydrochlorination

Table 8-2

CONFIRMATORY TESTS FOR ORGANOCHLORINE PESTICIDES (19)

Pesticide Class	Reagent or Reaction Type[1]
General	$CrCl_2$ reduction (26) KOH dehydrochlorination (46)
Hexachlorobenzene (HCB)	Base/alcohol KOH hydrolysis/diazomethane
BHC isomers	NaOMe/MeOH or GC alkaline precolumn
Cyclodiene insecticides	Comparison of 8 methods (D) 10 various reactions (D) BCl_3/2-chloroethanol (D/E) UV irradiation (D/E/H) H_2SO_4 or 60% KOH (E/M) _t_-BuOK/_t_-BuOH or $CrCl_2$ (E/M) Acid or base-Al_2O_3 microcolumn (C/E/H/T/M) Base-catalyzed intramolecular cyclization Silylation/acetylation (T/M)
Mirex	UV dechlorination
Kepone	KOH/esterification $LiAlH_4$/PCl_5
PCBs	$SbCl_5$ perchlorination
Chlorobiphenyls and PCP	Acetylation and butylation
DDT	Reduction and/or oxidation

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[1] Figures in parenthesis indicate the number of pesticides studied, letters indicate the particular pesticide(s) confirmed
C = chlordanes, D = dieldrin, E = endrin, H = heptachlor,
T = Thiodane (endosulfan) and M = and metabolites

Table 8-1 Continued

Parent Pesticide	Metabolite	Group Reacted	Derivative
Heptachlor	a) Chlordene	1) Allylic hydrogen 2) Double bond	1-Bromochlordene Chlordene epoxide
	b) 1-Hydroxychlordene	1) Allylic hydroxy 2) Double bond	Silyl ether Chloroacetate Epoxide
	c) 1-Hydroxy-2,3-epoxychlordene	1) Hydroxyl	Silyl ether
		2) Epoxide	Trihydroxy chlordane
trans-Chlordane	2-Chlorochlordene	Double bond or	Epoxide or
cis-Chlordane	3-Chlorochlordene	*gem*-dichloro group	hexachloro
cis- and *trans*-Chlordane	1,2-Dichlorochlordene epoxide	Chloro epoxide or *gem*-dichloro group	Chloroacetate or heptachloro
Endrin	Photo-endrin	*gem*-Dichloro	Pentachloro
Endosulfan	Endosulfan diol	Hydroxyl	Acetate or silyl ether

Table 8-3

CONFIRMATORY DERIVATIZATION REACTIONS FOR OCl PESTICIDES PUBLISHED SINCE 1975

Compounds Studied	Reagent (sensitivity)	Reference
HCB, PCBs and naphthalenes as interferences	Dechlorination in GC flow-through reactor containing carrier and H_2 gases + Ni catalyst (0.1-1 ng)	(20)
Heptachlor and its epoxide	UV light (0.01 ppm)	(21)
Mirex, PCB interference	Diethylamine-assisted photodecomposition of PCBs (0.2-5 ppm)	(22)
Phenoxyacetic acid herbicides	2,2,2-Trichloroethanol (0.1 ppb)	(23)
HCH, DDT, PCBs	MgO in GC microreactor	(24)
MCPA and MCPB herbicides	1-Bromomethyl-2,3,4,5,6-pentafluorobenzene (0.1 ppb)	(25)
10 Herbicidal acids	Pentafluorobenzyl bromide + K_2CO_3 catalyst (100-1000 pg)	(26)

A confirmatory technique related to chemical derivatization is ultraviolet degradation or photolysis (27, 28; Table 652-A of the FDA PAM). Degradation products arising from UV treatment of chlorinated insecticides and detected by EC-GC can provide identification of these pesticides (28) at 75-100 pg levels. Depending on the length of irradiation (often ca 10 minutes), all of the parent pesticide may not be degraded. Solvent and sample blanks should be run to prove if background is reacted as well. Isooctane is a good solvent because it is little affected by UV light.

Section 12,D,(1) of the EPA PAM gives details of a microscale alkali dehydrochlorination method for use in multiresidue analysis. This procedure produces derivatives for identity confirmation and provides supplemental cleanup for some troublesome extracts after Florisil chromatography. Section 651.12 of the FDA PAM describes the microscale alkali treatment method that is part of the AOAC official method for perthane. Table 651.1 lists the behavior of about 40 compounds under these reaction conditions. Alkali reactions carried out on a GC precolumn rather than in solution have proved advantageous in some instances (29). Section 12,D,(2) of the EPA PAM describes the confirmation of HCB in fatty tissues by formation of bis-isopropoxytetrachlorobenzene.

Section 11 of the Canadian PAM gives complete details for the following tests:

Pesticide(s)	Reagent
o,p'-DDT, p,p'-DDT, p,p'-TDE, methoxychlor	Sodium methylate
p,p'-DDT, endrin	Chromous chloride
Dieldrin, endrin	BCl_3 in 2-chloroethanol
Chlordane, heptachlor epoxide	K-tert butoxide/tert-butanol, silylation
Aldrin, heptachlor, p,p'-DDE	Chromic acid
Aldrin	m-Chloroperbenzoic acid
Endosulfan	Alcoholic KOH
Chlorophenoxy acid herbicides	n-Propanol
Captan	Resorcinol

A special two stage, mixed phase 180 cm column consisting of 165 cm of 4% OV-1/6% QF-1 and 15 cm of 3% OV-1/6% OV-225 at the injector end is recommended in the Canadian Manual for resolving HCB, BHC isomers, sulfur, and aldrin for confirmatory purposes, because they are not resolved on the 4% SE-30/6% QF-1 working column.

One method of differentiating PCBs from organochlorine pesticides is by treating the residues with an HNO_3-H_2SO_4 mixture. Organochlorine pesticides are destroyed whereas PCBs (and toxaphene) are unaffected. Other confirmation methods for PCBs are covered in Subsection 7A,E,e in Section 7 of this Manual.

b. OTHER PESTICIDE CLASSES

Residues of organophosphorus pesticides may be confirmed by alkaline hydrolysis followed by esterification of the resulting dialkyl phosphates to trialkyl phosphates (30). This procedure does not distinguish pesticides that produce the same hydrolysis product. According to McCully (31), the three most practical methods for confirmation of OP pesticides are oxidation to oxygen analogs (32), pentafluorobenzyl bromide derivatization of hydrolyzed phenols or thiophenols (33), and chromous chloride reduction (34). The sodium hypochlorite oxidation method has the widest applicability, but it suffers from low sensitivity, difficulty in analyzing the analog products, and inability to distinguish analogs originally present in samples. The $CrCl_2$ method is simple but applicable only to OP pesticides containing a nitro group. The pentafluorobenzyl bromide procedure is intermediate in scope. These and other reactions used to identify organophosphorus pesticides are listed in Table 8-4, along with information on triazine, carbamate, and urea pesticides. This table is from a review article (19) that gives the original references for these reactions. Table 8-5 contains a selection of more recent references.

Triazine herbicides have been confirmed by silylation, methoxylation (in sodium methoxide-methanol), methylation (CH_3I-NaH), and hydrolysis-DNFB reactions (35, 36); and linuron has also been confirmed by alkylation (with alkyl halide - NaH)(35).

8H SPECTROMETRY (SPECTROPHOTOMETRY)

Spectrophotometric methods for residue determination (quantitation) usually are not as sensitive or selective as GC or TLC, and for this reason they are not as widely used as in the early days of pesticide analysis before chromatographic methods were developed. The applicability of spectrometry is especially limited for multiresidue determinations or analyses of a parent compound, metabolites, and hydrolysis products.

Spectrometry can be very valuable, however, in conjunction with chromatography as a confirmatory tool, and it is this aspect that will be stressed in the following subsections.

8I VISIBLE, UV, FLUORESCENCE, AND PHOSPHORESCENCE

Very few pesticides are naturally colored, so a chromophoric group must be formed by a reaction or added through derivatization before most pesticides can be measured in the visible spectral region. The colorimetric method then becomes specific to the color forming group involved. The inferior sensitivity of direct and indirect visible spectrophotometric methods limits their usefulness for confirmation in human and environmental monitoring where residues are generally present at low concentrations.

The correlation between UV spectra and pesticide structure and the usefulness of UV spectrophotometry in confirming identification have been reviewed (51). Spectra-structure correlations can be of value to the analyst in identifying chromophores and therefore making confirmations, especially in conjunction with spectral information obtained by other methods, such as IR, NMR, and MS. In some cases, extinction coefficients (absorptivities) are sufficiently large to permit identifications at submicrogram levels. If a suitable absorption wavelength of a pesticide can be chosen that is free of interference from contaminants or solvents, UV spectrophotometry can be performed directly without sample purification and at a great saving of time. However, because absorption of UV energy is quite common for most organic compounds, rigorous cleanup may be required to remove any interferences that can absorb in the spectral region where the pesticide will be measured. The transparency of many functional groups (and often large segments of complex molecules) in the near UV spectral range imposes a limitation on interpretations of absorption bands in this region. Solvents must be carefully chosen to be transparent at the wavelengths absorbed by the pesticide. UV absorbing groups can be added by chemical derivatization methods, and this procedure has been used to detect pesticides by HPLC UV detectors and by TLC. UV spectra of 76 reference pesticides have been published (52). Visible and UV spectrophotometric methods for pesticides have been reviewed (53), including development of color by azo coupling and π complexing and recent instrumental developments.

If a pesticide is naturally fluorescent or can be made fluorescent by derivatization, fluorescence spectrophotometry is likely to be more

CONFIRMATORY TESTS FOR ORGANOPHOSPHORUS, TRIAZINE, UREA, AND CARBAMATE COMPOUNDS [19]

Pesticide Class	Compound Type	Confirmatory Test
Organophosphates	a) General	Hydrolysis/methylation
	b) Phenol-generating compounds	Hydrolysis/PFB ether formation
	c) Aryl-NO_2 and Aryl-CN containing compounds	Reduction ($CrCl_2$,$PdCl_2$, Zn/HCl)
	d) P=S compounds	Oxidation (to P = O)
	e) -NH and -NH_2 containing compounds	i) Alkyation (NaH/MeI/DMSO) ii) Deamination/methylation
	f) OH compounds (diazinon metabolites)	Silylation or alkylation
	g) Crufomate	UV dechlorination
Triazines	a) Chloro-*s*-triazines	i) Alkylation ii) Silylation iii) Methoxylation iv) Hydrolysis/DNP formation
	b) Hydroxy-*s*-triazines	i) Silylation ii) Alkylation iii) Chlorination
	c) Cyanazine and metabolites	Acid catalyzed cyclization
Carbamates and ureas	a) Intact compound	i) Acetylation ii) Silylation iii) Alkylation iv) Perfluorination*
	b) Phenol-generating compounds	i) Bromination ii) Chloroacetylation iii) Thiophosphorylation iv) Silylation v) Dichlorobenzene sulfonylation vi) DNT/DNP vii) Pentafluorobenzylation
	c) Amine-generating compounds	i) Iodination ii) Bromination iii) *p*-Bromobenzoylation iv) 2,4-DNP v) DNT vi) Pentafluorobenzylation (Amines in general)
Chlorophenoxy acids	a) Esters	i) Transesterification ii) Bromination

*includes trifluoroacetylation, pentafluoropropylation, and heptafluorobutylation

Table 8-5

CONFIRMATORY DERIVATIZATION REACTIONS FOR PESTICIDES
OF VARIOUS CLASSES PUBLISHED SINCE 1975

Compounds Studied	Reagent or Derivative (Sensitivity)	Reference
-NO_2-containing herbicides and fungicides	$CrCl_2$ reduction to -NH_2 followed by CCD-GC (0.5-1.0 ppm)	[37]
Organonitrogen fungicides and herbicides	Alkylation, methoxylation, trifluoroalkylation (0.1 ppm)	[38]
Carbofuran and metabolites	Heptafluorobutyric anhydride plus trimethylamine catalyst (10 pg)	[39]
OP pesticides	In-block methylation with THAM (µg levels)	[40]
Sulfoxide-containing pesticides	Trifluoroacetic anhydride (1 ppm)	[41]
Carbaryl	*N*-mono- and trichloroacetyl, and *N*-nitroso derivatives	[42]
Carbamate insecticides	Heptafluorobutyryl derivatives (0.1 ppm)	[43]
Dimilin (TH 6040)	Trifluoroacetyl derivative (0.02 ppm)	[44]
Thiabendazole	Pentafluorobenzyl chloride (0.01 ppm)	[45]
N-Aryl carbamates	Flash heater reaction with trimethylanilinium hydroxide (ng levels)	[46]
S-Containing carbamates	Trimethylphenylammonium hydroxide injected with compound into gas chromatograph (20 ng)	[47]
Carbamate and urea herbicides	Alkylation by NaH/CH_3I (0.1 ppm)	[48]
Dimilin (TH 6040)	Conversion to *N*,*N*'-dimethyl analog with NaH/CH_3I (0.25 ng)	[49]
Azodrin	Trifluoroacetylation (2 ppb)	[50]

selective and sensitive than either visible or UV absorption methods. Concurrence of fluorescence excitation and emission spectra between samples and standards, recorded either in solution or directly on thin layer chromatograms, has served as a valuable confirmatory aid for certain pesticides. Fluorescence characteristics are dependent on a number of experimental conditions which must be closely controlled, e.g., solvent and pH effects (54). Removal of naturally occurring fluorescent interferences from biological samples can pose serious cleanup problems. Fluorescence and phosphorescence methods for pesticides have been reviewed by Argauer (55) and the phosphorimetry of pesticides has been reviewed by Baeyens (56).

8J INFRARED (IR)

IR spectroscopy with micro sampling techniques is generally sensitive at the 1 μg level but has been used as low as the 0.1 μg level in some applications. It is thus considerably less sensitive than GC or TLC and cannot be used unless enough sample is available to provide a sufficient concentration of pesticides for IR observation. Sample extracts require a stringent cleanup procedure (e.g., partition plus column adsorption chromatography) plus additional purification either by GC or TLC. Thin layer spots are scraped and collected, and the pesticide is eluted from the adsorbent with an appropriate solution. Fractions can be collected from a gas chromatograph equipped with a stream splitter: a small percentage of the effluent stream goes to the detector for monitoring purposes while the remainder goes to a collecting device.

Potassium bromide (KBr) micro-pellet techniques using a pellet of 1-2 mm diameter are described in Section 12,E of the EPA PAM. These methods were developed by R. C. Blinn of the American Cyanamid Co. The key to their sensitivity is the ability to transfer the maximum amount of pesticide to a very small amount of KBr to be pressed into the micro-pellet. The equipment commonly used by Blinn for preparing micro-pellets by the syringe method is shown in Figure 8-B, and the technique for transfer of the sample-KBr mixture adhering to the syringe needle is pictured in Figure 8-C. The method using a commercially available "wick stick" is probably the most reliable and foolproof for preparing micro KBr pellets (Figure 8-D). The sample is applied to the wedge of potassium bromide, which is then dipped at its base into a volatile solvent. The solution migrates up the wedge to the tip where the solvent evaporates, and the compound becomes concentrated at the tip. The tip is then cut or broken from the wedge and pressed into a micro-pellet.

The Blinn microtechniques are sensitive and reliable, but considerable experience is required to prepare pellets with a minimum of contamination. They require the availability of a modern IR spectrophotometer including a beam condenser and microcells. Contamination from such sources as the sample, solvent, reagents, atmosphere, and handling is their major source of error. The same amounts of interferences which would be inconsequential for macro-sampling techniques become a significant percentage of a micro sample and contribute to the spectrum. Clean gloves should always be worn when preparing micro-pellets, and only purified solvents and reagents and carefully cleaned equipment should be used. Inevitable losses due to handling and processing require that the isolation procedure be started with sufficient sample to finally achieve a useable spectrum.

Another method which is in effect a micro-sampling technique involves scale expansion, or electronic amplification of the signal from the spectrometer. This method increases pen response without an increase in the sample concentration, but the response to all interferences and electronic noise is increased as well. All sources of interference must therefore again be minimized.

IR microtechniques have been reviewed by Blinn (57), including discussion of micro multiple internal reflectance. Advantages of internal reflectance include ease of applying (by dotting or streaking) sample to the surface of the reflectance plate (crystal), minimizing of interferences from handling and reagents, and ease of recovery after IR evaluation (samples made into pellets are essentially lost for further scrutiny). A disadvantage is lowered sensitivity compared to the KBr micro-pellet method. Sensitivity is increased by spreading a very thin film of sample over the effective sample area of a very thin reflectance plate. The multiple reflectance method has been applied to the identification of Thiram residues at 0.1 ppm on lettuce after extraction, Florisil chromatography, and TLC (58). An alternative micro-KBr technique with sensitivity levels similar to the method in the EPA PAM is detailed in the FDA PAM, Section 631.

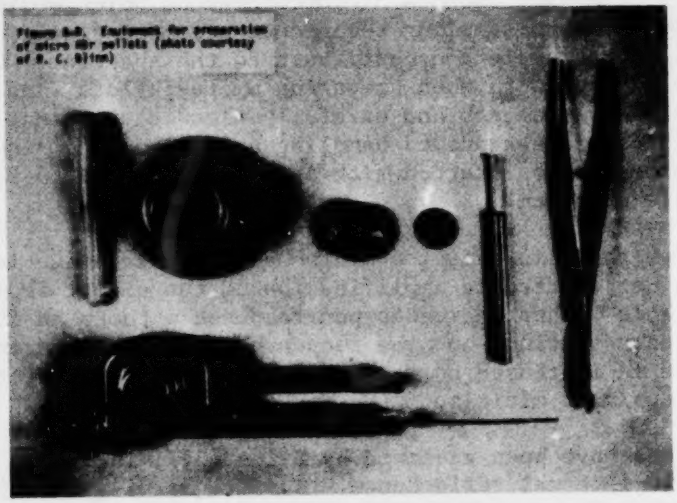


Figure 8-B. Equipment for preparation of micro KBr pellets (photo courtesy of R. C. Blinn)

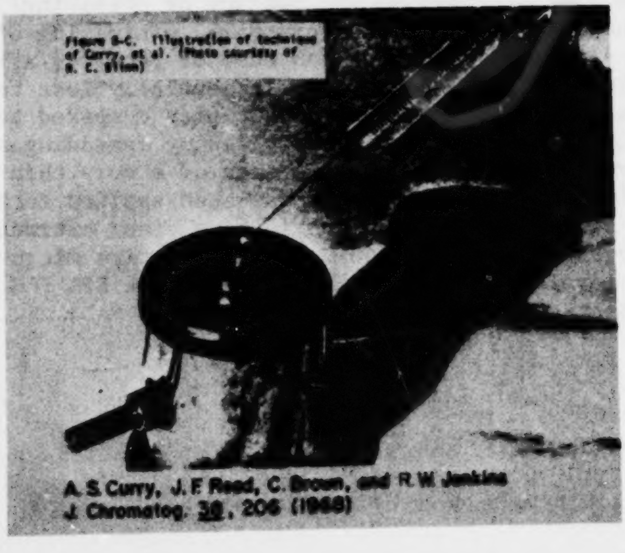
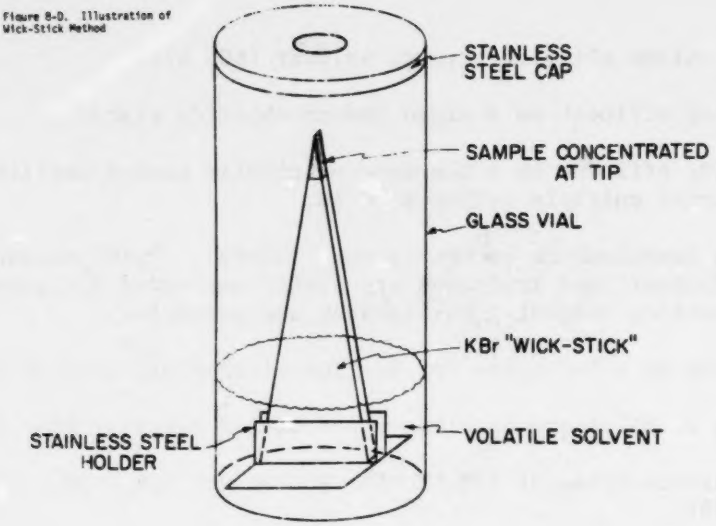


Figure 8-C. Illustration of technique of Curry, et al. (Photo courtesy of R. C. Blinn)

A. S Curry, J. F Read, C. Brown, and R. W. Jenkins
J. Chromatog. 38, 206 (1968)

Figure 8-D. Illustration of Wick-Stick Method



The FDA Manual (Section 632) also gives details of a qualitative micro procedure for collection of GC fractions directly on powdered KBr for IR confirmation. Interpretation of IR spectra from collected fractions must take into consideration the stability of the pesticide of interest to GC conditions. The analyst should be sure he is measuring the spectrum of unchanged pesticide rather than of a degradation product. In addition, the specificity of the GC detector will often obscure elution of interfering materials from the GC column, so that a fraction presumably containing isolated pesticide could be totally unsuitable for IR characterization. These interfering materials might be from the sample substrate or bleed or breakdown products from the stationary phase of the column packing. The column exit line should be heated at least to column temperature to the point of trapping, otherwise condensates resulting from previous samples may contaminate the trapped compound. Use of splitters at the column exit is usually necessary because of the high sensitivity (detectors would be overloaded by the µg quantities for IR) and the destructive nature of pesticide detectors.

Several different types of IR detectors directly coupled with gas chromatographs (59) have become available commercially but have not proven especially useful for pesticide residue work because of various disadvantages. Trapping procedures have been used almost exclusively, including the following methods:

a. Passing column effluent through solvent (60, 61).

b. Condensing effluent on a micro sodium chloride plate.

c. Condensing effluent on a thermo-electrically cooled capillary plate for internal multiple reflectance IR.

d. Trapping fractions on column packing (62-64). This procedure is very efficient, and fractions are easily collected for subsequent IR evaluation; reagent interferences are possible.

e. Collecting on a TLC plate for further cleanup prior to IR (65).

f. Trapping on Millipore or siliconized filter material (66, 67).

g. Using various types of liquid nitrogen or dry ice cold surface traps (68).

h. Using a cool or cold small i.d. tubing at the GC vent (69).

i. Trapping directly on KBr powder supported by pipe cleaner inside capillary tubing (FDA PAM, Section 632). This procedure is probably the most sensitive of any, tubes can be changed for each peak, and the technique is free of sources of interferences.

The choice of trapping procedure will depend on the amount of compound available, IR technique to be used, purity of the compound eluted from the GC column, and equipment available to the chemist.

IR spectra of over 400 reference pesticides have been published (70) to aid the analyst in matching spectra of unknown pesticides. The ASTM FIRST-1 computer search program (59) and similar computer retrieval systems aid in matching sample and reference spectra when standards cannot be easily chosen for a manual point-by-point comparison. Reference Raman spectra of OCl, OP, and carbamate pesticides were published (71).

An important recent development in IR analysis that is capable of sensitivity at subnanogram (72) residue levels is the Fourier transform (FT) or interferometric method. In FT-IR, a Michelson interferometer is used instead of the prism or grating and slits in a conventional spectrometer. The slitless spectrometer has an advantage in energy throughput, in addition to the so-called Fellget's or multiplex advantage that allows all wavelengths to be detected simultaneously throughout the spectral range. The signal to noise ratio increases with consecutive accumulation of scans and is proportional to the square root of the number of scans. Since each scan requires only a few seconds and instrument stability is high, many cumulative scans can be made on each sample. The fast scan capability is ideal for on-the-fly IR detection of GC effluents. FT-IR spectroscopy has at least an order of magnitude greater resolving power, greater wavelength accuracy, and a greater scan range than does conventional dispersion IR spectroscopy. There is also a much smaller image in the sample compartment without any special measures, making FT-IR ideal for microsamples. A dedicated minicomputer, in addition to the basic FT-IR optical equipment and detector, is required to collect, process, and store the data. FT-IR methodology and equipment have been reviewed (59). There is no doubt that much use will be made of FT-IR spectroscopy for pesticide determination and confirmation as the principles, techniques, and instrumentation become more familiar.

8K NUCLEAR MAGNETIC RESONANCE (NMR)

NMR spectroscopy has had only limited application in residue analysis because of its low sensitivity relative to other analytical methods, e.g., GC-MS, IR, and UV. Despite this drawback, it is one of the most valuable tools available for structural analysis and identity confirmation. Current pulsed Fourier transform NMR spectrometers (73) allow routine acquisition of useful data on as little as 10 µg of a proton NMR sample in a few minutes of experimental time. The NMR sensitivity of ^{13}C is lower; with current commercial instrumentation, a practical sample size is greater than 20 mg, although ^{13}C spectra of as little as 300 µg have been obtained on modified instruments (74). Useful information is provided by NMR in many areas relevant to the analysis of pesticides, their metabolites, and degradation products, such as identification and structural characterization, molecular geometries, conformations and stereochemistry, chemical kinetics and equilibria, complex formation and binding, and electronic charge distributions.

Residues of p,p'-DDT and p,p'-DDE isolated from adipose and liver tissue samples have been analyzed by NMR (75), with semi-quantitative determination of the relative concentrations of the pesticides. Included in NMR studies of the metabolism, binding, and degradation of pesticides are ^{1}H spectra useful for identification of p,p'-DDT (76, 77), p,p'-DDA (78), aldrin and dieldrin derivatives and other chlorinated pesticides (79), rotenoids (80), and dithiocarbamates (81). Other ^1H reference spectra of organophosphorus (82), diphenylmethane (DDT type) (83), and carbamate (84) pesticides have been published and are useful for identity confirmation. The application of NMR to pesticide analysis has been reviewed (73, 85, 86).

Carbon-13 NMR spectra have been published for α-BHC (87) and for several chlorinated biphenyls (88, 89). Studies of technical chlordane components (90), Mirex (91), and Kepone and its photo-products (92) contain ^{13}C NMR data useful for confirmation and structural characterization. Chlorine nuclear quadrupole resonance spectrometry has been used to study the structures of several chlorinated pesticides including BHC, aldrin, endrin, endosulfan, and dieldrin (93-95). ^{31}P-NMR chemical shifts have been correlated with structures of some organophosphorus pesticides (96), and ^{31}P Fourier transform NMR has been used for the determination of malathion at ppm levels (97).

8L MASS SPECTROMETRY (MS)

The mass spectrometer is a very sensitive spectroscopic tool for pesticide residue analysis, providing useful data on ng or less material. Ions are produced from neutral sample molecules and are then sorted according to their mass-to-charge ratio. The mass spectrum is a record of these different ions and their relative abundance. A mass spectrum is usually quite characteristic of an individual pesticide, sometimes even providing data that will differentiate geometric isomers. Pesticide identifications can be made by matching the mass spectrum of an unknown sample with the mass spectrum of a known material. This comparison is especially valuable because it is based on many different peaks characteristic of the unknown compound. Careful inspection of key fragment ions in the spectrum can also assist in elucidating the structure of an unknown compound even without comparison to a reference material. These procedures are feasible with both low and high resolution spectrometers, but the exact elemental compositions of the parent ion (if obtainable) and fragments provided by high resolution MS is a valuable aid to the analyst.

a. MS INSTRUMENTATION AND OPERATION

(1) Introduction

Five components are common to most mass spectrometers: the inlet system, the ion source, the mass analyzer, the detector, and the readout system. In addition, a vacuum must be maintained throughout the spectrometer from inlet to detector so that ions formed in the source will not be lost from collisions with atmospheric gas molecules. A sample is introduced to the ion source where it is ionized via the inlet system. The function of the inlet system is to transfer the sample from a high pressure (i.e., 1 atm) region into the high vacuum of the spectrometer without seriously unbalancing the spectrometer operation. The generated beam of ions is focused and separated in the mass analyzer according to the m/e ratios. The detection system senses the mass-separated ion beams, and the readout device translates the signal provided by the detection system into an output that can be interpreted by the analyst. Excellent references for pesticide residue analyses by MS have been written by Ryan (98), Safe and Hutzinger (99), and Skinner and Greenhalgh (100).

(2) Inlet Systems: Combined GC-MS

Samples may be introduced directly into the ion source with a direct insertion probe assembly. For example, the sample is loaded into a short length of melting point capillary, placed in the heater well at the end of a probe, and inserted to within a few millimeters of the ion source through a vacuum lock that maintains a vacuum-tight arrangement. The temperature is then raised until the sample vaporizes and a spectrum is obtained. Techniques for trapping GC fractions for introduction into an independent mass spectrometer have been published (63, 101-103), and pesticides cleaned-up on and eluted from thin layer plates have also been studied by MS.

For impure samples such as biological extracts, the gas chromatograph of a coupled GC-MS instrument serves as an efficient inlet system for introduction of samples into the spectrometer. The resolution provided by gas chromatography offers extra sample cleanup in addition to any partition and liquid column chromatography steps. Temperature and sometimes flow rate programming have proven useful for achieving high chromatography resolution with the combined instrument. Column bleed can be a serious problem in GC-MS, since bleeding liquid phase is also detected by the mass spectrometer and contributes spurious ions to the analytical spectra. Carefully conditioned, low bleed columns that are stable at high temperatures should be used whenever possible. Other approaches that alleviate problems from column bleed include use of a short, bleed-absorbing column placed between the analytical column and the GC-MS interface, programming the flow rate of the carrier gas, and computer subtraction of background resulting from the bleed (104).

Compatability of the gas chromatograph and mass spectrometer is a problem because of the large volume of carrier gas eluting from the chromatograph and the need to operate the spectrometer at high vacuum (10^{-5} - 10^{-6} mm Hg). In the simplest approach, the two instruments are connected directly and a large pumping system is used to maintain the required vacuum in the mass spectrometer. This approach has been used successfully with GC columns having flow rates up to ca. 20 ml/minute. Introduction of samples from packed columns into the mass spectrometer requires removal of most of the carrier gas by means of an interface between the two instruments. At the same time, as much sample as possible should be retained so that the gas flowing into the spectrometer is enriched in sample. Three basic types of sample enriching devices or separators have widespread use in modern GC-MS systems, namely effusion, jet, and silicone membrane; each has its own advantages and limitations. In all cases, some carrier gas enters the source along with the sample molecules, and broadening of GC peaks by the interface may occur. In practice, most separators convey only 20-40 percent of the sample in the GC effluent to the mass spectrometer. The theory and operation of separators is described in detail by McFadden (105). The mass spectrometer in a combined instrument must be able to scan through an appropriate mass range, e.g., from mass 10 to mass 800, in a small fraction of the time that it takes to elute the peaks from the gas chromatograph.

Combined LC-MS

GC-MS is sometimes limited by the volatility or heat sensitivity of the compounds under study. To circumvent these difficulties, various methods of interfacing a high pressure liquid chromatograph with a mass spectrometer have been explored (106-111). As of this writing, only one manufacturer offers a commercial liquid chromatograph-mass spectrometer (LC-MS) interface (112). This interface consists of a continuous belt that accepts the LC effluent in a chamber at atmospheric pressure and then sequentially passes it beneath an infrared heater and through two vacuum locks into a vaporization chamber. Under optimum conditions the LC solvent is evaporated from the belt by the heater and vacuum locks, leaving only a deposit of the sample on the belt. The vacuum locks also accomplish the transition from atmospheric pressure to the vacuum system of the mass spectrometer. In the vaporization chamber a second heater volatilizes the sample in front of a nipple leading into the ion source. A third heater cleans the belt before its return to the atmospheric chamber via the vacuum locks.

This interface is able to accommodate most commonly used organic LC solvents at optimum flow rates varying from about 0.2 to 1.5 ml/min. The use of water as an LC solvent generally requires an LC effluent splitter if reasonable LC flow rates are to be used, since the maximum capacity of the interface for water appears to be about 0.1 ml/min. The LC-MS system has been successfully applied to the analysis of a large number of carbamate pesticides (112).

The field of LC-MS is still under development. Other commercial LC-MS interfaces are anticipated in the near future.

(3) Ionization Processes

The most widely used ionization source is the electron impact (EI) type wherein gaseous molecules are ionized by electrons emitted from a glowing filament. These positive ions are accelerated into the analyzer section. The EI source is relatively stable and easy to operate, and has high ionization efficiency. The overall quantity of positive ions and the nature of the fragmentation process depend on the energy of the ionizing electron beam.

At low electron energy levels (0-20/electron volts or eV), much of the ion current tends to be carried by unfragmented molecular ions. However, the absolute intensity is relatively low. At higher energy levels, fragmentation and rearrangement are more prevalent, and the ion current is much higher. Molecules are often cleaved to such an extent that the molecular ion is absent from the mass spectrum or is of very low intensity. Because mass spectra are more reproducible when compounds are ionized by 60-80 eV electrons, most mass spectrometers are operated in this energy range. It is noteworthy that the EI source produces mass spectra that are quite repeatable among instruments and distinctively characteristic of the compounds being ionized. This has led to the collection of large libraries of mass spectral data with which unknown spectra can be compared. Such comparisons often permit rapid identification of the unknown pesticide.

Chemical ionization (CI) spectra are obtained by adding methane, helium, or other reagent gas (at relatively high pressures of about 1 mm Hg) to the sample either as the GC carrier gas or after removal of the GC carrier gas by the separator. In the latter case, the CI reagent gas is introduced into the MS just ahead of the point at which the effluent enters the ion source, or into the source itself. Electrons produce reagent gas ions that subsequently ionize sample molecules by chemical reactions, e.g., proton transfer, hydride abstraction, ion attachment. The mass spectra obtained with CI are quite different from those formed on electron impact and are, in general, simple, easy to interpret, and complementary to electron impact spectra for unequivocal pesticide confirmation. Although CI usually provides molecular ion (or $M^+ + 1$ or $M^+ - 1$) peaks of high intensity, a study (113) of a series of chlorinated and organophosphorus pesticides found no molecular ion produced from electron impact or chemical ionization. CIMS has sensitivity at least as good as that of EI (114) and offers the advantage of allowing characterization of a sample's chemical reactivity through the choice of the reagent gases. In addition to methane and helium, isobutane, hydrogen, argon-water, ammonia, nitric oxide, and other gases have been used successfully to produce CI spectra. The positive and negative methane (115) and isobutane (116) CI mass spectra of selected chlorinated insecticides of several types and methane positive ion CI data for 29 organophosphorus insecticides and metabolites (117) have been determined and published. Negative CIMS with methylene chloride reagent gas was the basis of a multiresidue screening procedure of OCl residues in environmental substrates at 1 ng levels (118).

Field ionization (FI) involves passing a gaseous compound between an anode (usually a thin wire or sharp blade) and a cathode. An extraordinarily high electric field, approximately 10^8 V/cm, is impressed on the anode, permitting (according to one theory) valence electrons of the sample to "tunnel" to the metal of the wire or blade. A positive ion results, which can be separated according to mass-to-charge ratio and detected. This is a relatively low energy or soft ionization method that often produces enhanced molecular ion intensities and a cleaner spectrum for compounds with poor thermal stability. Fragmentations are less prevalent and different from those observed in normal EI spectra. The FIMS of a number of pesticides has been studied (119), and FIMS has been combined sequentially with HPLC for the determination of trifluralin (120).

Field desorption MS is a modification of FI in which the sample is applied directly to the anode. As with FIMS, field desorption depends on application of very high electric fields to this anode. Sample molecules in contact with the anode desorb as ions into the source, where they are separated and mass analyzed. Like FI, field desorption is a soft ionization process usually resulting in minimal sample fragmentation. Unlike FI, field desorption has no requirement that the compound be volatile prior to ionization. Mass spectra can be obtained for samples that are thermally unstable or have no appreciable vapor pressure. Strong molecular ion peaks are produced for most pesticides (113) including highly polar pesticides and metabolites (121). However, assignment of molecular ions in biological samples can be complicated by the presence of $(M + H)^+$, $(M + Na)^+$, or other ion adducts.

A novel method with unsurpassed sensitivity involves generation of ions with an atmospheric pressure ionization (API) source. This source uses ^{63}Ni on gold foil to produce electrons that can interact with nitrogen and water passing through the ionization chamber at atmospheric pressure. Preheated carrier gas enters the API source just behind the sample injection port. Both gas and sample pass through the ^{63}Ni source block where ionization reactions take place. Just beyond the chamber is a small aperture through which the ions pass on their way to being mass analyzed and detected. With certain samples, this source generates more ions for a given quantity of sample molecules than any other ion source; this is reflected in the reference to the API mass spectrometer as the "femtogram machine" (122). The ^{63}Ni source has been replaced by a corona discharge (123), producing identical API mass spectra and limits of detection but a greater dynamic response range. The API source is not yet commercially available and has not been evaluated for residue analysis, but it obviously has great promise in this regard.

A discussion of 13 methods for ionization of organic compounds in MS has been published, including detailed consideration of chemical ionization and field ionization and pesticide spectra (124).

(4) Mass Analyzer Systems

Low resolution magnetic analyzer systems depend on deflection of the ion beam in a magnetic field. The magnetic field classifies and segregates the ions into beams, each of a different m/e. To obtain the mass spectrum, the magnetic field is varied and each m/e ion from light to heavy is successively brought to focus on the exit slit. Such analyzers are referred to as single- or direction-focusing analyzers. High resolution instruments have an analyzer region with an electrostatic sector for velocity or kinetic energy focusing plus a magnetic sector for separation of fragments according to m/e ratio.

Quadrupole analyzers are based on mass separation in a radio frequency (RF) electric field. This field is established on a set of four precision parallel, usually circular, rods, with both a d.c. voltage and an RF alternating voltage being applied to these electrodes.

Ions are accelerated gently (5-30 V) into the analyzer or filter region and begin to oscillate between the rods of rapidly alternating polarity. At a given d.c. and RF level, ions of a specified m/e value undergo stable oscillation and pass through the length of the analyzer tube to the detector. Ions of lower or higher mass will undergo ever increasing oscillations that eventually result in their striking one of the rods. The spectrum is obtained by sweeping the applied RF alternating frequency and measuring the detector current as a function of time.

(5) Resolution

Resolution describes the performance of the mass analyzer in terms of its ability to separate ions of different masses from one another. Resolution is expressed in numerical form by the equation $\Delta M/M$, where M and $M + \Delta M$ are mass numbers of two neighboring peaks of equal intensity in the mass spectrum. The criterion for resolution is a relative height of the valley between peaks of 10 percent, with each peak contributing 5 percent to the valley. For example, an instrument would have a resolution of 100 if two peaks with a mass difference of 1 part in 100 (e.g., m/e 100 and 101) were resolved to the 10 percent level. Low resolution mass spectrometers typically show maximum resolution values between 300 and 1000 while high resolution instruments are capable of attaining resolutions well in excess of 10^4. The advantage of a high resolution spectrometer is the capability of resolving ions with very little differences in mass and obtaining the molecular ion and fragment ion masses accurately to 0.001 mass units or better. Exact masses are determined using a computer coupled to the mass spectrometer or by peak matching known marker peaks and unknown peaks on an oscilloscope (125). Once the exact mass of a key ion (often the molecular ion) is known, the elemental composition or formula of the fragment is obtained, again by using a computer or by consulting tabulations of the masses of different combinations of atoms indicated to be present by the mass spectral pattern or prior information about the unknown sample.

Resolutions of the order of 1000 are attainable with low resolution magnetic and quadrupole analyzer designs, although single-focusing magnetic analyzers can attain higher resolutions with an extreme decrease in sensitivity due to the narrow slits that must be used. Resolution in excess of 8000 is considered high, since this is the amount usually necessary to resolve most mass doublets. The extra focusing added in a high resolution mass spectrometer reduces the overall number of ions traversing the instrument, thus reducing the overall sensitivity. To overcome such a reduction, the mass range is usually scanned at a slow rate. To minimize the effects from slow scanning and decreased sensitivity, only as much resolution as is necessary to perform the required analysis should be used, since the accuracy of an exact mass measurement is independent of resolution as long as any mass doublets are separated.

Descriptions of Finnigan and Hewlett Packard GC-MS systems and detailed operating parameters are given in Section 8M. References (98, 99, 101, 126, 127) review methods and applications of MS and combined GC-MS to pesticide residue analysis, and references (105, 128-130) give a more general survey of GC-MS instrumentation and principles.

b. EXAMPLES OF GC-MS CONFIRMATION

Figure 8-E shows the electron capture gas chromatogram obtained by injection of an aliquot of the 6 percent ethyl ether Florisil column eluate from cleanup of a human adipose tissue extract (131). Figure 8-F shows the total ion current chromatogram of the same eluate from GC-MS. Although the curves are drawn to different scales and are not directly comparable, it is evident that many more compounds are identifiable in the latter because of the superior specificity of the mass spectrometer. In general, chromatograms traced by the total ion monitor are similar, but not necessarily identical, in response and sensitivity to that traced concurrently by a flame ionization detector. Differences exist in sensitivities to some compounds, and broadening occurs in some peaks in the interface to the mass spectrometer.

FIGURE 8-E ELECTRON CAPTURE CHROMATOGRAM OF HUMAN ADIPOSE TISSUE EXTRACT, 6% ETHER FLORISIL COLUMN ELUATE

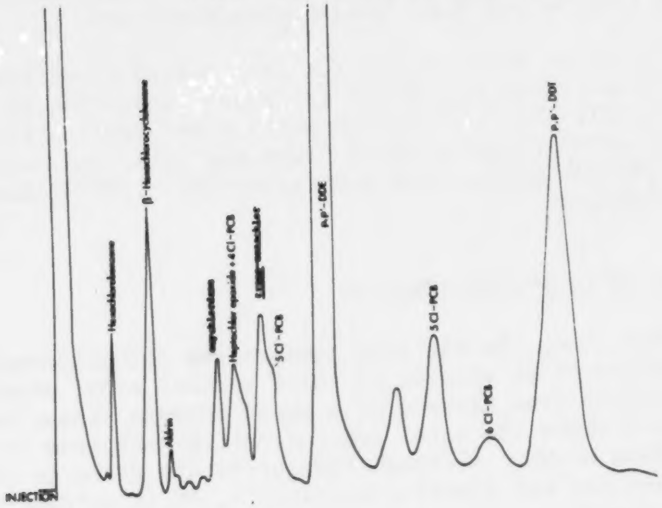


FIGURE 8-F TOTAL ION CURRENT CHROMATOGRAM OF SAME HUMAN ADIPOSE TISSUE EXTRACT

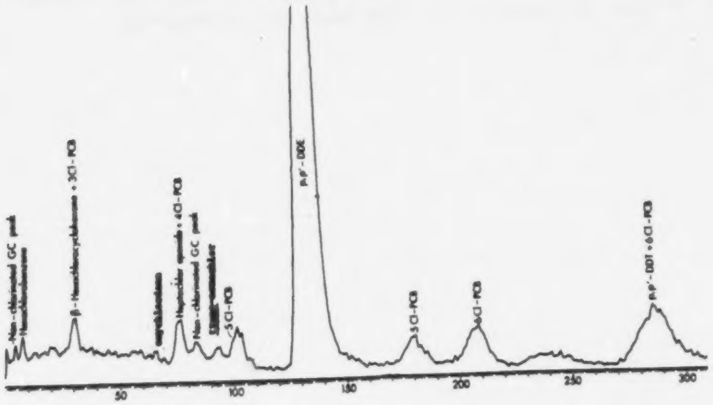


Figure 8-G is the mass spectrum of the key p,p'-DDE GC peak evident in both chromatograms in Figures 8-E and 8-F. The molecular ion (M^+) peak at 316 amu and the characteristic fragments at 281, 280, and 246 would confirm the tentative identification of this peak as p,p'-DDE obtained from comparing the retention time of the GC peak to standard tables. Figure 8-H illustrates the total spectrum of standard p,p'-DDE for comparison to Figure 8-G.

The identification of pesticides from their mass spectra is often complicated by the obscuring of low mass ions by impurity fragments, especially in biological extracts. For this reason, extra cleanup of extracts may be needed for GC-MS as compared to GC alone. For example, alkaline hydrolysis has been used for the 15 percent ethyl ether Florisil column eluate, while additional column adsorption cleanup (e.g., alumina plus Florisil columns) or use of silica gel rather than Florisil initially has been successful for the 6 percent ethyl ether eluate. Gel permeation chromatography has also been successfully applied to the 6 and 15 percent fractions (132).

c. THE MASS SPECTROMETER AS A GC DETECTOR

There are a number of ways to use the mass spectrometer as a sensitive and selective GC detector. These procedures require that the analyst know what compound or compounds he is looking for and are not applicable to totally unknown samples.

Selected ion monitoring (SIM), also called multiple ion detection (MID) or multiple ion selection (MIS), involves automatic, continuous monitoring of a few ions of different masses. Tracings of the selected masses are recorded simultaneously as rapid switching is accomplished in the spectrometer to bring each ion into the detector in turn for a short period of time. Simultaneous recording of one or several compounds can be achieved, with characterization of each being based on the formation of one or more selected ions (133). To use SIM effectively, one should know the kind of compound sought and its MS characteristics. Sensitivity of detection for SIM can sometimes be extended to the sub-picogram range, which is considerably more sensitive than conventional scanning because of the longer sampling time at each selected mass. Sensitivity for a particular compound reflects the extent of fragmentation and fraction of the total ion current carried by the selected ions. Identification of compounds can be improved by exact mass measurement (e.g., to 0.001 amu) of the specified ion, but only at the expense of sensitivity.

FIGURE 8-G MASS SPECTRUM OF p,p'-DDE PEAK;
Ions below 200 not shown

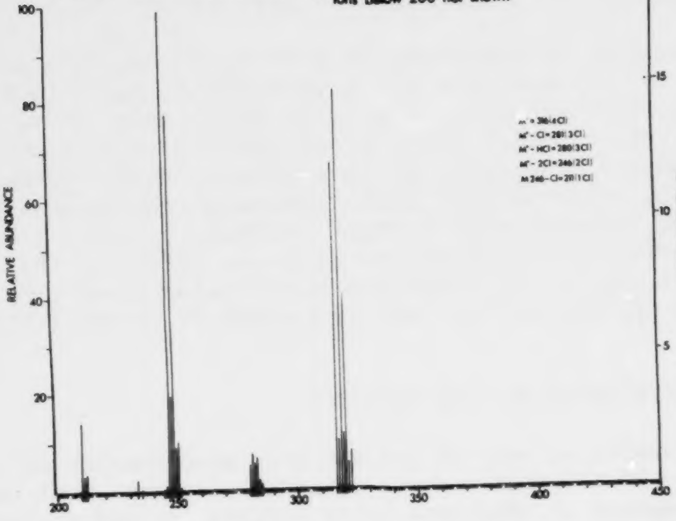
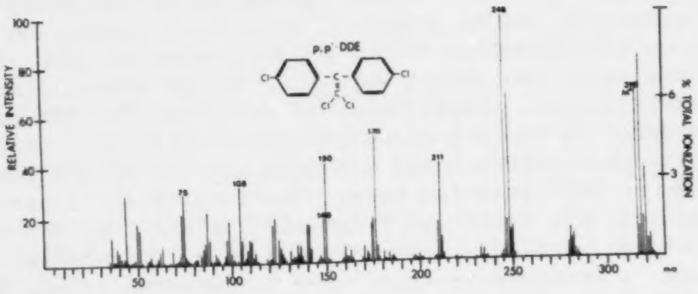


FIGURE 8-H TOTAL MASS SPECTRUM OF p,p'-DDE



Total ion current plots (TICP) cannot be generated by the SIM technique because data from only certain masses are collected. A plot of the change in ion abundance as a function of time using abundances measured by SIM is termed a selective ion current profile (SICP). These and other nomenclature problems have been discussed along with suggested remedies by Budde and Eichelberger (129). Compounds not resolved by gas chromatography can still be detected with certainty if their molecular (or other selected) ions can be resolved by SIM. Recording the masses and relative intensities of several ions formed from a single pesticide can increase the certainty of compound identification. SIM has been applied to the detection of organophosphorus insecticides (134) and to carbofuran and metabolites in crops (135).

Repetitive scanning through a narrow mass range generates quantifiable spectral envelopes from several ions at once. This procedure, generally sensitive at low ng levels, has been applied to pesticide analysis (136).

Reagent ion monitoring is an interesting variation of single ion monitoring, wherein the intensity of reagent ions used in a chemical ionization source is monitored as a function of time. The intensities of reagent ions decrease when they react with material eluted from the GC column, providing a chromatogram that is distinctive from those produced by other detectors (130).

d. COMPUTERIZATION OF GC-MS

Combination of a computer with a GC-MS system can serve several very useful functions.

(1) The computerized GC-MS data acquisition system permits rapid processing of information from complex sample mixtures. The mass spectra of specific compounds in the mixture can be experimentally obtained and automatically matched with a library file of standard mass spectra. Computer control of data acquisition may enable the operator to generate relatively complex scanning procedures. For example, different mass ranges may be sampled for different time periods, masses may be sampled for times related to the intensities being measured, or several discontinuous mass ranges may be sampled.

(2) Column bleed and other background can be conveniently subtracted by the computer.

(3) Continuous repetitive scans can be made during the entire chromatographic separation, for example, a spectrum can be scanned every 2-4 seconds. In a typical GC-MS run, several hundred to more than a thousand mass spectra may be acquired in this way, each one being a complete spectrum over the mass range selected.

All spectra are stored, and chromatograms may later be reconstructed by the computer by summing and plotting the total ion current detected in each scan but excluding carrier gas ions or other interfering ions. Total ion profile chromatograms obtained resemble those traced in real time by a conventional total ion monitor of a magnetic deflection spectrometer.

(4) The computer can trace the intensities of selected characteristic masses from among the great quantity of data acquired by continuous repetitive scanning. The resulting mass chromatograms or extracted ion current profiles (EICP) (129) resemble the single or selected ion profiles described earlier and permit compounds and spectra of interest to be located and the appropriate spectrum to be retrieved and plotted. EICPs have an advantage over SIM in that large numbers of ion profiles and complete spectra can be examined rapidly after only one chromatographic separation, but this computerized acquisition of repetitively scanned spectra is of considerably lower sensitivity (as much as 10^4) than SIM because of the longer integration time characteristic of the latter method (129). Reference (127) illustrates computer-generated individual ion chromatograms.

The limited mass range chromatogram (130) is a variation of mass chromatography that has proved especially valuable in the determination of polychlorinated hydrocarbons. In this technique, the computer sums ion intensities (collected from repetitive scanning) through a limited mass range as a function of scan number or time. (The procedure has also been termed selected ion summation analysis or SIS.) For example, the molecular ion cluster of Mirex, due to the contributions of ^{37}Cl from each of the 12 chlorine atoms, is spread over a range of more than 27 amu. Instead of treating a single ion (e.g., $C_{10}{}^{35}Cl_{12}{}^{+}$, nominal m/e 540), the entire cluster can be summed to provide increased sensitivity with some sacrifice in specificity. The method has been used to identify dieldrin and HCB residues in lake trout (137).

(5) Quantitation of peak areas in the selected ion profiles and ratios of these peaks can be provided. Computer coupled GC-MS equipment is extremely expensive, highly qualified personnel is needed for its operation and maintenance and to interpret data, and a significant

amount of "down-time" is to be anticipated because of its complexity. Computerized data acquisition and processing for magnetic instruments, quadrupole instruments, and selected ion monitoring have been described (98).

e. APPLICATIONS OF GC-MS TO PESTICIDE ANALYSIS

Reference spectra and fragmentation data for pesticides of several types and for related chemicals have been published (96, 99, 138, 139). Applications of GC-MS include confirmation of the 1-naphthyl chloroacetate derivative of 1-naphthol (a carbaryl metabolite) extracted from urine (140); 2,4-D, 2,4,5-T, and 2,4,5-TCP in urine (141, 142); organophosphorus pesticides in blood and urine (143, 144); multiple chlorinated insecticides in human adipose and liver tissue (131, 132, 145) or foods (146); toxaphene in human and biological samples (147); Kepone in human and environmental samples (148); chlordane-related residues in human samples (131); and thiabendazole and 5-hydroxythiabendazole in animal tissue (on-column methylation plus SIM) (149). An important application of GC-MS has been mutual determination and identification of PCBs in the presence of chlorinated pesticides (150). Insecticides mixed with PCBs have been identified at levels below 10 ng without complete separation on a GC column by peak monitoring MS as described earlier (151). GC-MS has been successfully applied to the detailed analysis of complex pesticide mixtures, such as technical chlordane (152). Pesticides and PCBs have also been identified by GC-MS using chlorine isotope ratios to reconstruct chromatograms that are characteristic for the number of chlorine atoms found in repetitive-scan spectra (153). Special MS and GC-MS techniques that have been applied to the analysis of simple and complex pesticides in a variety of sample substrates include selected ion monitoring (154, 155), glass capillary techniques (156), field ionization (157), and field desorption MS (158). Methods have also been developed for the determination of carbamates by combined liquid chromatography-mass spectrometry (112). References (98, 126, 159, 160) contain further reviews of applications to residue analysis.

8M QUALITY ASSURANCE OF GC-LOW RESOLUTION MS

This section reviews procedures to be followed for quality assurance of data derived from the mass spectrometer in the identification, confirmation, and quantitative determination of chlorinated insecticides, PCBs, hexachlorophene, and PBBs in human tissues and fluids. These methods were developed at the Health Effects Research Laboratory, U.S. EPA, Research Triangle Park, NC (161) for use in the EPA National Human Monitoring Program for adipose tissue and serum samples. The procedures assure interpretable mass spectra of the highest experimentally obtainable quality for compound identification as well as quantitative accuracy when monitoring ion intensities (as by selected ion monitoring). The specific pesticides of current interest are the following:

- *o*,*p*'-DDT
- *p*,*p*'-DDT
- *o*,*p*'-DDE
- *p*,*p*'-DDE
- *o*,*p*'-DDD
- *p*,*p*'-DDD
- α-BHC
- β-BHC
- Lindane (γ-BHC)
- δ-BHC
- Aldrin
- Dieldrin
- Heptachlor
- Heptachlor epoxide
- Endrin
- Mirex
- Oxychlordane
- *trans*-Nonachlor
- Polychlorinated biphenyls
- Hexachlorobenzene
- Polybrominated biphenyls
- Polychlorinated terphenyls

a. INTRODUCTION TO THE QUALITY ASSURANCE PROCEDURES

Correct identification of organic pollutants from gas chromatography-mass spectrometry (GC-MS) data require valid mass spectra of the compounds detected. This is independent of the actual method of interpretation of the spectra; i.e., an empirical search for a match within a collection of authentic spectra or an analysis from the principles of organic ion fragmentation. A properly operating and well tuned GC-MS is required to obtain valid mass spectra.

The purpose of the following procedure is to permit a check of the performance of the *total* operating computerized GC-MS system. Thus, with a minimum expenditure of time, an operator can be reasonably sure that the GC column, the enrichment device, the ion source, the ion separating device, the ion detection device, the signal amplifying circuits, the analog to digital converter, the data reduction system, and the data output system are all functioning properly.

An unsuccessful test requires the examination of the individual subsystems and correction of the faulty component. Environmental data acquired after a successful system check are, in a real sense, validated and of far more value than unvalidated data. Environmental data acquired after an unsuccessful test may be worthless and may cause erroneous identifications. It is recommended that the tests be applied often on a working system, especially when there is a suspicion of a malfunction.

The procedure is written for a low resolution mass spectrometer such as the Finnigan 3200 or the Hewlett Packard 5930A quadrupole-type mass spectrometer, equipped with an automated data system such as the Finnigan 6000 or Hewlett Packard 5933A system. (See Subsections g and h for descriptions and operating parameters of these instruments.) However, the test is clearly and readily adapted to any GC-MS system by suitable modification of the detailed procedure.

There is a special need to closely monitor the performance of the quadrupole mass spectrometer. Unlike the magnetic deflection spectrometer, the active ion separating element of a quadrupole spectrometer (the rods) is directly contaminated during operation and after prolonged operation is subject to severely degraded performance. Since degraded performance usually affects the high mass region first, the test includes high mass end criteria. High quality, high mass data are important since many environmentally significant compounds have molecular and fragment ions in the 300-500 amu range.

A quadrupole mass spectrometer, which has been tuned to give a reference compound spectrum that meets the criteria of this test, will, in general, generate mass spectra of organic compounds that are very similar, if not identical, to spectra generated by other types of mass spectrometers. Thus, quadrupole mass spectra will be directly comparable to spectra of authentic samples in collections that have developed over the years, mainly from magnetic sector mass spectrometers.

Assurance of mass spectral data is obtained through a set of two levels of functionality tests. The first test requires establishment of production, dispersion and detection of ions from a reference compound, perfluorotri-n-butylamine (PFTBA). Relative peak heights are adjusted to conform to the known electron impact spectrum, with a slight biasing toward increased transmission of ions higher than m/e = 200, which are not commonly interfered with by tissue component fragments.

The second test of the GC-MS combination requires injection of a known low-level standard sample while the operation is under computer control. This is followed by periodic verification of the quality of spectra compared to spectra of known ideal quality. Chemical compounds used may be bis(perfluorophenyl)phenylphosphine (or decafluorotriphenyl phosphine, DFTPP). Another set of compounds commonly used are aldrin and heptachlor epoxide. Heptachlor epoxide is useful as a representative member of the important chlordane series of pesticides and, more generally, because the M-Cl ion, six-chlorine isotope cluster beginning at 351 m/e allows a test of sensitivity and resolution at a very useful mass, not provided for by PFTBA or many other mass calibration compounds, but quite relevant to pesticide work. The appearance of the 351 m/e cluster may be examined at 100, 10 and 1 ng levels, as instrument sensitivity requires, with respect to appearance of the six-chlorine cluster versus statistical appearance. Resolution of ^{13}C isotope peaks and relative abundance versus the 81 m/e peak may also be determined. Aldrin, injected as a GC retention time test, also has its mass spectrum routinely compared against the literature spectrum with respect to correctness of chloro cluster statistics, sensitivity, and relative appearance of high and low mass fragment ions. The retention time of heptachlor epoxide relative to aldrin (1.59 ± 0.02) on a 1.5% OV-17/1.95% OV-210 column may also be determined, along with GC column resolution. This test has the advantage of providing a full functionality evaluation of the GC-MS system, including sensitivity, data system acquisition, and recall of spectra.

b. QUALITY ASSURANCE PROCEDURES

(1) Using PFTBA (3M trade designation: FC-43) standard:

(a) Check on oscilloscope and/or light beam oscillograph that 69, 131, 219, 264, 414, 502, and 614 m/e ions are present and in reasonable relative abundance according to the following tabulation:

General Desired Appearance of the Mass Spectrum of PFTBA

Mass (m/e)	Relative Abundance %
69	100.0
100	24.3
114	9.5
119	20.3
131	70.3
219	68.0
264	16.2
414	5.4
426	2.7
502	2.7
614	0.3

^{13}C Isotope Abundance Checks, Percentage Ratio of Ion Signal Abundances

(70)/(69) = 1.1%

(219)/(220) = 4.4%

(502)/(503) = 10.3%

(b) Tune mass spectrometer as required, with respect to resolution, optimum peak shape, sensitivity, and minimum mass falloff (refer to appropriate instrument manual for instructions).

(c) Calibrate data system and verify the calibration by examining a PFTBA spectrum acquired under data system control (refer to appropriate data system manual for programs).

(2) Run aldrin and/or heptachlor epoxide and examine the reconstructed total ion chromatogram and mass spectra.

(3) Perform DFTPP test (optional).

(4) Go on to sample runs.

c. PREPARATION OF ALDRIN AND/OR HEPTACHLOR EPOXIDE STANDARDS

Primary standards of aldrin and heptachlor epoxide can be obtained from the Pesticide Repository, Health Effects Research Laboratory, EPA, Research Triangle Park, NC.

Carefully weigh out 20 mg of the pesticide and dissolve in 100 ml of n-hexane (pesticide quality or equivalent) in a volumetric flask. Keep this stock solution under refrigeration. Replace every 6 months.

Prepare a working standard of 20 ng/µl concentration by diluting 1 ml of the stock solution to 10 ml in a volumetric flask. These working solutions should be replaced at least monthly.

d. PREPARATION OF DECAFLUOROTRIPHENYL PHOSPHINE (DFTPP) STANDARDS

Prepare a stock solution of DFTPP at 1 mg/ml concentration in acetone (pesticide quality, or equivalent). This stock solution has been shown to be 97+ percent stable after 6 months, and indications are that it will remain useable for several years. Dilute an aliquot of the stock solution to 10 µg/ml concentration in acetone. The very small quantity of material present in very dilute solutions is subject to depreciation due to adsorption on the walls of the glass container, reaction with trace impurities in acetone, etc. Therefore, this solution may be useable only in the short term, perhaps 1-3 weeks.

e. QUALITY ASSURANCE TEST

(1) Adjust the GC column flow to normal operational level (e.g. 30 to 45 ml/min) and set the desired oven temperature (e.g. 185°C). The parameters should be adjusted to provide at least four spectral scans during the elution of the aldrin, heptachlor epoxide, or DFTPP standard.

(2) Set mass spectrometer at normal or high sensitivity as desired.

(3) Calibrate the instrument.

(4) Inject 40 ng of aldrin and/or heptachlor epoxide (or 20 ng of DFTPP) on the GC column and note the time (or start stopwatch).

(5) After the solvent passes through the analyzer and the vacuum has recovered, turn on the ionizer and start scanning.

(6) Note the exact retention time of the standard as it elutes from the column. This retention time can be used as a daily check of the condition of the GC column and separator by comparing the values. The retention times should not vary significantly from day to day under identical operating conditions.

(7) Terminate the run, turn off the ion source and electron multiplier, and reconstruct the gas chromatogram.

(8) Select a spectrum number on the front side of the GC peak as near the apex as possible and select a background spectrum number immediately preceding the peak.

(9) Plot or display the mass spectrum and compare against a reference spectrum. The spectrum obtained on the test system should contain ion abundances within limits given for the key ions in the following tables. Sensitivity is considered adequate if 40 ng or less of either aldrin or heptachlor epoxide and 20 ng or less of DFTPP provide good mass spectra.

Reference Aldrin Mass Spectrum
(5-chlorine cluster check)

m/e	Abund. (%)
261	61.5
262	4.7
263	100.0
264	7.8
265	65.0
267	21.1
269	3.4
271	0.2

$\frac{(261)}{(66)} = 45\%$

Reference Heptachlor Epoxide Mass Spectrum
(6-chlorine cluster check)

m/e	Abund. (%)
351	51.2
352	5.6
353	100.0
354	11.2
355	81.2
357	35.2
359	8.5
361	1.1
363	0.06

$\frac{(351)}{(81)} = 47\%$

Reference Mass Spectrum of DFTPP

Mass	Ion Abundance Criteria
51	30-60% of mass 198
68	Less than 2% of mass 69
70	Less than 2% of mass 69 (1.1% theoretical)
127	40-60% of mass 198
197	Less than 1% of mass 198
198	Base peak, 100% relative abundance
199	5-9% of mass 198 (6.6% theoretical)
275	10-30% of mass 198
365	1% of mass 198
441	Less than mass 443
442 ($M^{+\cdot}$)	40-60% of mass 198 -- this ion is very sensitive to spectrum number chosen and condition of equipment. If greater than 60%, equipment is OK if all other criteria are met.
443 (M+1)	17-23% of mass 442 (19.8% theoretical)
444 (M+2)	1.86% (theoretical)

f. PROTOCOL FOR ANALYSIS OF SAMPLES

(1) Sample Collection

Samples of human adipose tissue are obtained through cooperating medical pathologists and medical examiners at hospitals in cities selected according to a proportionate, stratified-random design. The conterminous 48 states were divided into 9 census divisions, according to the 1970 census of the United States. A city within each census division was selected from those already participating in the National Human Monitoring Program as the collection site for special projects.

Blood sera samples are collected throughout the U.S. by means of a cooperative arrangement between EPA and the U.S. Public Health Service. The PHS program, called the Health and Nutritional Examination Survey II (HANES II), provides blood specimens from a probability sample of persons 12 to 74 years old, along with various medical and nutritional parameters and some information regarding pesticide use by the individuals sampled. The blood is drawn into evacuated ampoules, allowed to clot, and centrifuged, and the serum is decanted into a clean vial.

(2) Clean-up

Tissues are normally extracted and cleaned up according to a modified Mills-Olney-Gaither procedure (Subsection 7A) by laboratories under contract to the National Human Monitoring Program. Concentrated extracts, corresponding to the 6 percent and 15 percent ethyl ether in petroleum ether fraction from the Florisil cleanup column, are then sent to the ACB/HERL-RTP for GC-MS analysis. Composite samples, comprising 100-500 individual samples, require additional cleanup before GC-MS analysis. The usual method of choice is gel permeation chromatography (GPC) as described in Subsection j. Blood sera samples may or may not need GPC cleanup.

(3) Analysis

After cleanup, samples are concentrated by removal of solvent at room temperature under a gentle stream of nitrogen. The final volume is usually 100 µl, but it may be smaller if levels of compounds sought are particularly low. Qualitative analysis is performed in the electron impact (EI) mode. Aliquots of 5 to 50 µl are co-injected with aldrin (e.g., 250 ng in hexane) as an internal standard into the GC-MS system. A total ion chromatogram is generated, and retention times relative to aldrin are determined for each component of interest. Mass spectral data are recalled from the computer for each component of interest and analyzed against reference mass spectra obtained from various literature references (e.g., 96, 99, 126, 145, 159), through the Cyphernet™ reference library, or generated from authentic laboratory standards. Relative retention times are also compared to those of the reference material for further confirmation. After identification, quantitative analyses are usually performed by selected ion monitoring (SIM). An authentic reference sample is used for direct comparison (See Subsection i). Identification may be further confirmed by chemical ionization GC-MS where available (Subsection h).

g. DESCRIPTION OF HEWLETT PACKARD GC-MS SYSTEM AND OPERATING PARAMETERS

The Hewlett Packard 5930A quadrupole-focusing mass spectrometer is interfaced with a Hewlett Packard 5700A gas chromatograph and Hewlett Packard 5933A data system. The mass spectrometer is operated in the electron impact (EI) ionization mode typically at 70 eV electron energy and 120 µamps filament emissions with a target current of 100 µamps. Selected ion monitoring (SIM) runs are normally made at 100 µamps and high electron multiplier voltages. The ion source temperature is usually maintained at 180°C and the mass filter temperature at 110°C. Ions are detected by a Bendix continuous dynode electron multiplier with voltages varied between 1.5 and 3.0 Kv, as required for sufficient signal strength. Data are collected, stored, and plotted by the 5933A data system. Selected ion monitoring is controlled by a suitable program provided by the manufacturer and by the data system. Data output also involves the use of a light beam oscillograph, as well as plotted mass spectra and reconstructed total ion chromatograms from the data system.

The gas chromatograph is often equipped with 1.8m x 2mm (i.d.) glass columns packed with 1.5% OV-17/1.95% OV-210 or 3% OV-17 on 80-100 mesh Gas Chrom Q. The GC inlet temperature is normally 200°C and the helium carrier gas flow rate 40 to 45 ml/min. Isothermal runs are typically made at 180° to 190°C, while temperature programming is usually from 80°C (2 min) to 210°C (or higher) at 8°C/min. The transfer line to the mass spectrometer is maintained at 210°C, and the silicone membrane separator is kept at 180°C.

Detailed operating instructions for the Hewlett Packard mass spectrometer, gas chromatograph, and data system are given in the appropriate manufacturer's operating manuals.

h. DESCRIPTION OF FINNIGAN GC-MS SYSTEM AND OPERATING PARAMETERS

(1) Equipment

The Finnigan Model 3200 quadrupole mass spectrometer is equipped with both electron impact and chemical ionization vacuum consoles. Each vacuum console is interfaced to a dedicated Finnigan Model 9500 gas chromatograph. Mass spectra and chromatographs may be obtained "manually" by the use of a light beam oscillograph and strip chart recorder, respectively. Alternatively, the collection and storage of data may be placed under the control of a Finnigan Model 6100 data system. The data system is capable of data collection in either scanning or a selected ion monitoring (SIM) mode.

Detailed operating instructions may be found in the appropriate Finnigan manuals.

(2) GC Conditions

Column: 150 cm x 2 mm i.d. glass column silanized before packing.

Packing: 1.5% OV-17/1.95% OV-210 on 80-100 mesh Gas Chrom Q.

Freshly packed columns are baked at 275°C for at least 48 hrs with a carrier gas flow. The oven temperature is then reduced to 250°C. The column is then treated with five 20 μl injections of a silating reagent such as Silon-CT at 10 minute intervals. The testing of standards for column evaluation is begun not less than 30 minutes after the final injection.

Carrier: Carrier gas flow is regulated to approximately 20 ml/min. Helium is used as a carrier gas for electron impact ionization studies and for those chemical ionization studies in which the reagent gas is not suited for gas chromatography. Methane is the routinely used chemical ionization reagent gas, and it is suitable for gas chromatography.

Column oven: In order to obtain sharp, resolved chromatographic peaks for most of the mixture components, the column oven is normally operated under a temperature program. The initial run is generally made with a 1 min isothermal hold at 150°C followed by a programmed increase of 2°/min to 275°C.

Injector:	200-225°C
Transfer line:	200°C
Separator oven:	250°C

Subsequent runs are made under conditions which, in the opinion of the operator, will enhance regions of interest in the chromatogram.

(3) MS Conditions

For qualitative studies, the mass spectrometer is operated in an electron impact (EI) ionization mode under control of the data system. The operator variable controls are set to the following values:

Preamp:	10^{-8} amp/volt
Electron multiplier:	2 kV
Emission current:	0.5 ma
Electron energy:	70 eV
Analyzer heater:	50°C

The lens, repeller, ion energy, and resolution controls are adjusted on a day-to-day basis to obtain an optimum spectrum of the reference compound perfluorotri-*n*-butylamine (PFTBA or FC43) as per the detailed instructions in the Finnigan "3200/3300 GC/MS Systems Operation Manual".

Chemical ionization with methane as the reagent gas is often used for confirmation. Operator variables are as follows:

Preamp: 10^{-8} amp/volt
Electron multiplier: 2 kV
Emission current: 1.0 ma
Electron energy: 110 eV
Analyzer heater: 70°C
Source temperature: 120°C
Source pressure: 900-1000 microns

The instrument is optimized for CI studies as per the instructions in the Finnigan "Chemical Ionization System: Models 3100E, 3200E, 3300E; Operation and Maintenance Manual".

(4) Data Systems Parameters

The data system is generated in a scanning mode for qualitative studies. Scan time is held to approximately 5 sec/scan in order to retain as much chromatographic resolution plus sensitivity as possible. In order to achieve this, the scan is generally restricted to 450 amu. For chlorinated compound analyses (e.g., PCBs, chlordane, DDE, etc.), the following parameters are used:

Mass range: 100-150, 151-230, 231-430, 431-550
Integration time: 3, 6, 12, 18 milliseconds/amu
Seconds per scan: 5
Threshold: 1
Maximum run time: 90 minutes
Instrument range setting: E

For quantitative analyses, selected ion monitoring is normally used. The exact parameters are sample and component dependent. The masses to be monitored are chosen at the time of the analysis.

i. QUALITY CONTROL OF SELECTED ION MONITORING DATA (FOR HEWLETT PACKARD SYSTEM)

Operation in the selected ion monitoring mode is verified by injection of known levels of the compound to be determined and examination of the resulting signal levels. Tuning of such instrumental parameters as the peak width repeller voltage and ion focus is usually required, and high voltage on the electron multiplier must be used for maximum sensitivity. Usually several test SIM runs are required to obtain sensitivity. In addition, the following precautions are to be routinely exercised:

(1) Run blank checks involving all solvents, glassware (evaporators, etc), and microliter syringes to verify the absence of the compound in question. Blanks may commonly consist of ca 10 ml of solvent evaporated to 0.1 ml in an evaporator tube in a stream of nitrogen, followed by injection of 20 µl into the GC-MS system.

(2) Study the sample using scanning mass spectrometry in an attempt to ascertain if interfering ions are present from sample components that may tend to "swamp" or interfere in retention time with the compound to be monitored. The scanned spectra obtained add to the qualitative evidence of the presence of the compound.

(3) Bracket samples with levels of known standards.

(4) Co-inject standards of approximately equal levels with the sample to be monitored and observe the signal to see if it is doubled. Also determine whether the identity is consistent with the GC retention time.

(5) GC column resolution may decrease as sequential fat injections are made. This may be partially corrected by removing the glass wool plug from the on-column injection port of the gas chromatograph. However, the GC column will usually have to be changed as a study progresses.

(6) Intersperse blank solvent injections after standard injections in order to verify the absence of "hang-up" of any of the compounds to be monitored in any component of the system, with subsequent release by the solvent "burst" of the following injection.

Careful attention to the above protocol will produce results as "real" as can be obtained by low resolution GC-MS. Caution must be exercised, however, because low resolution SIM data combined with a correct retention time is not always sufficiently specific to define the compound qualitatively. For example, tetrachlorodibenzo-*p*-dioxin monitored ions may be interfered with by PCBs.

Readers interested in further information on this topic are referred to reference (154).

j. GEL PERMEATION CHROMATOGRAPHIC CLEANUP OF ADIPOSE TISSUE

(1) Theory

Gel permeation chromatography (GPC) is a form of liquid chromatography by which compounds are separated according to molecular size. It is particularly useful in separating very large molecules such as lipids and cholesterol found in adipose tissue samples from the smaller molecules of pesticides, PCBs, etc. The method is as effective as the MOG procedure for cleanup in pesticide residue analyses (162) and has the added advantages that removal of fat is more complete and recoveries of pesticides are nearly quantitative. Hence, it is the ideal choice for GC-MS analyses, where maximum detectability of pesticides is needed and minute quantities of lipid materials can cause serious interferences.

Porous polymer beads (e.g., Bio-Beads SX-3) are used as gel particles and organic solvents (e.g., toluene, ethyl acetate, or cyclohexane) are used for the mobile phase. The elution process is very simple (isocratic only); the same solvent system is used for column preparation, elution, and washing. Macromolecules cannot permeate the porous gel and are rapidly eluted or "dumped" from the column. Molecules which can enter the pores of the beads are temporarily retained to greater or lesser extents depending on their molecular volumes. Hence, large-volume pesticides such as Mirex elute first (in this case, following shortly after cholesterol), while small-volume pesticides such as HCB elute last. Since molecular volume rather than molecular weight dictates the order of elution, all equatorial β-BHC elutes after the other BHC isomers.

(2) Equipment

The gel permeation chromatograph is an AutoPrep Model 1001 (Analytical Biochemistry Laboratories, Inc., Columbia, MO), equipped with a 2.5 cm (i.d.) x 60 cm glass column (Chromaflex^{R} R-422350/6025, Kontes, Vineland, NJ, or equivalent) packed with 200- to 400-mesh Bio-Beads SX-3 (Bio-Rad Laboratories, Richmond, CA).

(3) Column Preparation and Operation

(a) Prepare a slurry of ca 60 g of Bio-Beads SX-3 in pesticide quality (or equivalent) toluene-ethyl acetate (1:3 v/v). This will be sufficient to pack a column about 25 cm long.

(b) Add small volumes of resin and solvent to the column. Each addition of resin must be in contact with enough solvent to swell the resin before the next addition.

(c) After the resin is transferred to the column, compress the gel to approximately 25 cm, allowing solvent to flow out of the column exit.

(d) Add only toluene-ethyl acetate (1:3 v/v) to the solvent reservoir. Addition of other solvents to the system via sample introduction will change the gel swelling ratio and must be kept to a minimum (i.e., $<5\%$ v/v of aliquot injected).

(e) Install the column and start the pump. The pump operating pressure should be 5-7 psi (not to exceed 10 psi).

(f) Adjust the pumping rate to approximately 5 ml/min with the pump vernier control valve.

(g) Set the timer to collect for 20 minutes and check the actual pumping rate.

(h) The GPC elution pattern of the pesticides of interest should be established for standards before introduction of biological samples into the gel permeation chromatograph.

(4) Procedure for GPC Cleanup

(a) Start up the GPC instrument and elute the column with toluene-ethyl acetate (1:3 v/v) until it is purged of entrained air.

(b) Introduce ≤ 1 gram of sample in 8 ml of eluting solvent into the sample introduction valve. Rotate to the next sample loop and introduce the next sample. After the last sample is loaded, rinse the sample valve with clean solvent.

(c) Set "dump" and "collect" cycles to previously established rates for elution of the pesticides of interest (usually 100-125 ml and 125-225 ml, respectively).

(d) For multiple sample cleanup, an appropriate "wash" cycle of ca 50 ml toluene-ethyl acetate (1:3 v/v) should be included after every sample.

8N BIOLOGICAL METHODS

Bioassay techniques, which include insecticidal activity, enzymatic, and immunological methods, have been described as providing an independent criterion of identity when combined with GC, chemical reactions, etc. (2). These methods, which depend on the measurement of a physiological response of a test organism induced by exposure to the pesticide, have advantages of simplicity and sensitivity but are relatively non-specific so that their utility for confirmation is rather poor. The insect bioassay technique has been reviewed (163).

Specificity of enzyme inhibition is greatly enhanced by combination with TLC for detection and confirmation of organophosphate and certain carbamate pesticides. The R_F value plus biological response provides important identity information at levels typically in the range of 500 pg to 10 ng for these compounds.

80 POLAROGRAPHY (VOLTAMMETRY)

The use of polarography as a confirmatory test is described in Section 12,F of the EPA PAM and Sections 640 and 641 of the FDA PAM. Procedures and applications of polarography for both identification and determination of pesticide residues have been reviewed (164, 165).

Polarographic identification of a pesticide residue is based on the determination of the peak potential of the unknown in a cleaned-up extract, and comparison with the potential of about the same amount of a reference standard under identical conditions. As a check, addition of the standard compound to the unknown should result in an increase in the wave height but not appearance of another wave. Mixtures can be identified if the peak potentials of the components are sufficiently separated. Trapped GC fractions may be subjected to polarography to confirm identifications based on retention times. Instrumentation for such modern voltammetric techniques as fast sweep oscillography provides sensitivity comparable to colorimetry. Pesticides not containing an oxidizable or reducible functional group can be made amenable to polarography by formation of a suitable derivative (e.g., nitro, halogen, carbonyl, etc).

Most polarographic studies have been applied to phosphorus-containing insecticides such as parathion, diazinon, malathion, and carbophenothion. A collaborative study confirmed the usefulness of single sweep oscillographic polarography for identifying such residues in non-fatty foods (166). Thirty-eight herbicides have been studied by single sweep derivative polarography (167), methylcarbamate insecticides by AC polarography and cyclic voltammetry (168), and urea herbicides by anodic polarography (169). Published voltammetric reduction potentials for about 100 organochlorine insecticides, PCBs and naphthalenes (3 electrode potentiostat, DMSO solvent) are a useful aid in identification of residues (170). Parathion and related insecticides and metabolites were polarographically determined in blood without extraction (171). The polarography of 1,3,5-triazines (172), propachlor herbicide (in soil) (173), dithiocarbamates (174), dinitroaniline herbicides (175), thiourea-containing pesticides (176), and trifluralin (in soils) (177) has been reported.

8P MISCELLANEOUS CONFIRMATORY METHODS

a. CARBON SKELETON CHROMATOGRAPHY

Carbon skeleton chromatography (CSC) is useful in characterizing insecticide residues in amounts down to 5-100 ng. Apparatus for CSC consists of a precolumn containing a hot (ca 300°C) catalyst attached to a GC equipped with a flame ionization detector (available from National Instruments Laboratory, Rockville, MD). The compound to be identified is injected directly on the catalyst bed (e.g., 1% Pd on 60-80 mesh Gas Chrom P) and is swept over the bed by hydrogen carrier gas. Nitrogen is introduced through the normal instrument inlet so that the detector yields optimum response. While in the precolumn, all functional groups are stripped from the compound, and any multiple bonds are saturated. The resulting hydrocarbons are carried into the chromatographic column where they are separated and identified by their retention characteristics relative to standards. This identification method, which is in effect a derivatization procedure, has been applied to heptachlor, heptachlor epoxide, chlordane, aldrin, endrin, DDT and its analogs, and carbaryl. Sufficient residue must be available for the method to be of value. Techniques, applications to many pesticide classes, and characterization of products of CSC (as well as some other precolumn reaction confirmatory methods) have been reported by Beroza and co-workers (178-181) and Asai et al (182, 183). Identification of 5-10 ng amounts of polychlorinated biphenyls, terphenyls, naphthalenes, dioxins, and dibenzofurans in biological samples has been demonstrated (184).

b. FRAGMENTATION PROCEDURES

GC fragmentation procedures are similar to CSC except that the reaction in the precolumn decomposes the pesticides, yielding characteristic fragment peak patterns or fingerprint chromatograms helpful in making identifications. A palladium catalyst at 300°C (182) and reagents such as Na_2CO_3, CuO, $CdCl_2$, $AlCl_3$, and $K_2Cr_2O_7$ at 240°C (185) have been applied to chlorinated and OP insecticides with EC detection of the reaction products.

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Section 9

MAINTENANCE, TROUBLESHOOTING, AND CALIBRATION OF INSTRUMENTS

The EPA Pesticide Analytical Manual, Appendix I, contains comments on the maintenance and repair of instruments primarily intended for laboratories which are part of the EPA or have contractural arrangements with EPA allowing them to make use of the electronic repair facility located at Research Triangle Park, N. C. The Instrument Shop in RTP is equipped to handle repairs, modifications, and calibrations on various gas chromatographs, recorders, and GC detectors.

A detailed treatment of instrumental servicing and calibration is beyond the scope of this Manual. Some general comments and a few selected topics of special interest will be covered, however.

9 A DAILY OPERATIONAL CONSIDERATIONS FOR GAS CHROMATOGRAPHIC INSTRUMENTATION

(a) Is the proper carrier gas connected into the system?

(1) Is the tank capable of maintaining the desired flow for an eight hour work period without going below 500 psi tank pressure?

(2) Is the tank output pressure normal (40-50 psi recommended)?

(b) What is the detector condition, temperature, flow, background profile (BGS), and polarizing voltage?

(c) Is the electrometer operating properly, and is it zeroed properly? Is bucking ability adequate? What is the noise level?

(d) Has the programmer temperature remained constant? Is the final hold control in a safe position to avoid accidental overheat operation?

(e) Does the purge system operate smoothly? Will it be used on this day's operation? When was it last checked for leaks?

(f) Is the oven damper closed? Does it function?

(g) Are all temperature set controllers functioning properly and is the voltage to the load (heaters) stable? Is the oven at proper temperature?

(h) Has the recorder been checked for proper speed, zero, gain level, dead band, ink supply, and sufficient paper? How long has it been since calibration?

(i) Are the septums, "O" rings, and glass injection inserts in good condition? When were they last replaced?

(j) Is the pyrometer reading correct and are the compensator mercury batteries good? When were they replaced last?

9 B CHECK LIST WHEN INSTRUMENTAL REPAIR IS INDICATED

A systematic check-out routine is recommended to determine whether instrumental service or repairs can be completed "in-house" during normal instrument "off-time", or if outside help is required. Results should be written down as the check-out is completed so that full information can be transmitted for service. Serial numbers and EPA numbers of the equipment involved should be recorded as part of this information. Although the check-out presented is specifically for a Tracor MT-220 gas chromatograph, the steps are illustrative of the kind of routine that can be established for any analytical instrument.

Erratic operation of the instrument in day-to-day use is often an indication that serious trouble is imminent. Keep in mind the type of detector, column, carrier gas, and temperature range being used. Recall, or preferably, consult the "instrument log" to determine when the instrument was last serviced "in-house" (e.g., detector or column change) and if the difficulties arose directly or shortly after such service.

Check List

(a) Is there proper insulation packing in the detector compartment? Improper packing can lead to variation in signal due to ambient temperature changes.

(b) Are any wires exposed, shorted, or loose?

(c) Check proper location and readings of thermocouples and resistance thermometers; are they fully seated?

(d) Plumbing leaks should be checked for at full operating pressure before heat is applied, and again at operational temperatures.

(e) Is carrier gas pressure correct? Tank pressure should be greater than 500 psi and output pressure 40-50 psi.

(f) Are flow indicators functioning smoothly? Are they steady? Does the float go smoothly through its entire range?

(g) Are panel indicator lamps correct? Temperature programmer indicators in particular should be observed for smooth transition from heat to cool cycle.

(h) Are switches set properly, ie., oven damper in closed position for heating modes?

(i) Does the recorder respond correctly to attenuation or input changes, bucking, zero, heat rise or cycling?

(j) Has signal to noise level increased? Refer to previous chromatograms obtained at like settings.

(k) Has the EC background profile decreased appreciably?

(l) Is the oven door secure? Also check rear access door.

(m) Are all units such as the EC power supply, recorder, electrometer, etc., plugged into the proper power source?

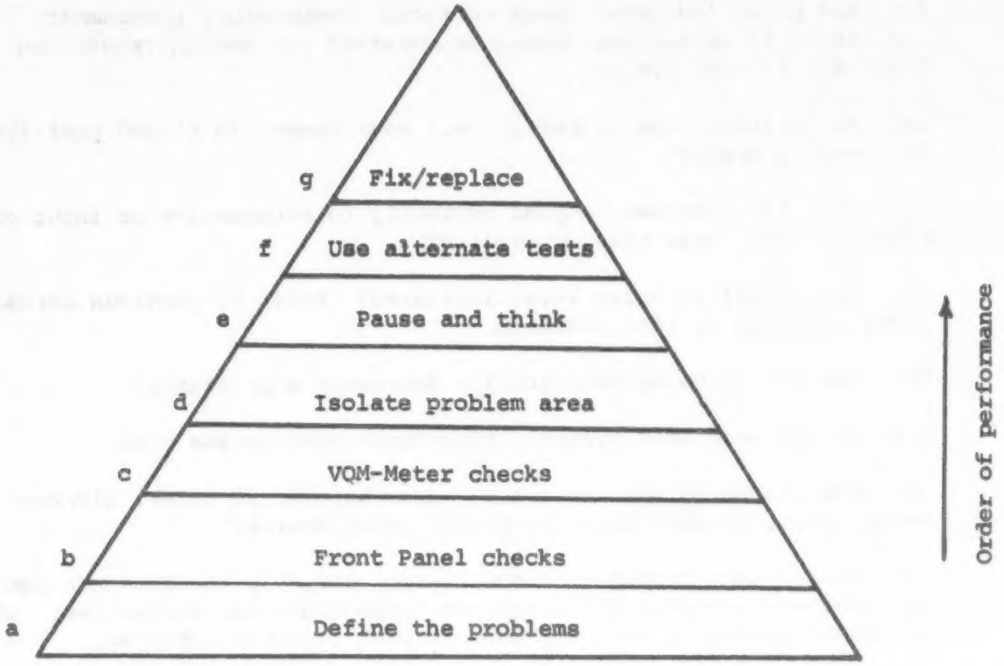
(n) Check that thermometers and thermocouples in the detector compartment are attached to the proper temperature set controllers and pyrometer points if the detector has been recently serviced.

(o) Is the column properly conditioned and is it the best one to be used in terms of stationary phase selection, efficiency, and freedom from bleed?

(p) Check color coding of circuits for accuracy. The correct color codes should always be maintained to facilitate servicing. Further tests may be recommended by service personnel to confine the problem to a particular section of the instrument. The various modules are interconnected by cables and wires, and these should be disconnected in progression from the detector toward the recorder. A volt-ohm-milliammeter with a loading factor of 20 K ohms per volt is a necessary item to have on hand when checking and servicing electronic and electrical components.

9 C GENERAL APPROACH TO TROUBLESHOOTING

The following pyramid approach represents a logical, general method for instrumental troubleshooting


g Fix/replace
f Use alternate tests
e Pause and think
d Isolate problem area
c VOM-Meter checks
b Front Panel checks
a Define the problems
Order of performance

(a) Write down the symptoms or difficulties observed. Diagnosis should include observations of instrument settings.

(b) Examine the problem by using the controls, meters, and lights on the instrument.

(c) Use a VOM to check voltages or resistances.

(d) Bracket the system path - electrical, mechanical, flow, and chemical. The overall system and local systems must be considered.

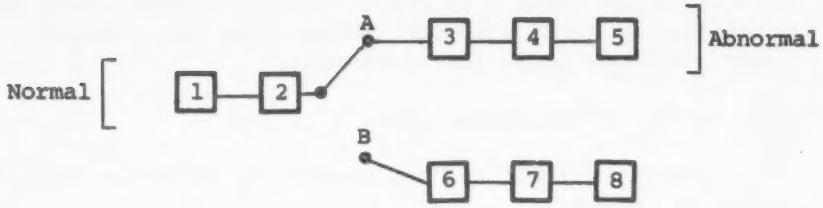
Isolation principles:

Before starting: Read the instrument manual - understand the instrument, know how to use test equipment, and understand test results.

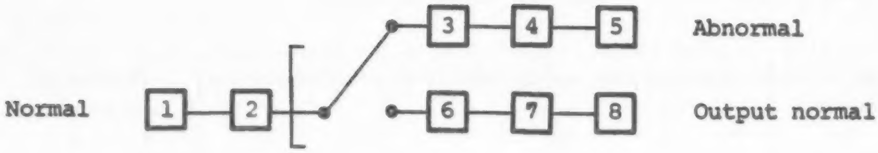
What to do first: Use logical approach, bracket the problem to a system, bracket the area in the system, use checks to isolate faulty area.

Signal paths: Use a block diagram and locate where in the signal path normal and abnormal output occurs.

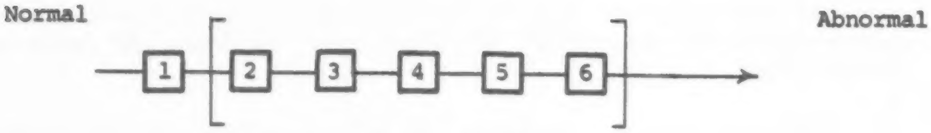
e.g.,


A
Abnormal
Normal
1
2
3
4
5
B
6
7
8

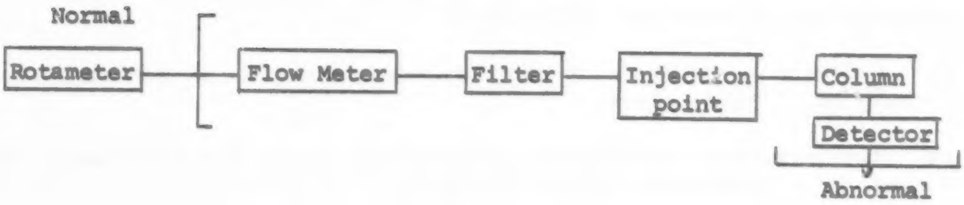
or


3
4
5
Abnormal
Normal
1
2
6
7
8
Output normal

Linear path - make your check in the middle of a bracketed linear path - "Half Split" Rule.


Normal
Abnormal
1
2
3
4
5
6

Observe and measure for normal/abnormal readings


Normal
Rotameter
Flow Meter
Filter
Injection point
Column
Detector
Abnormal

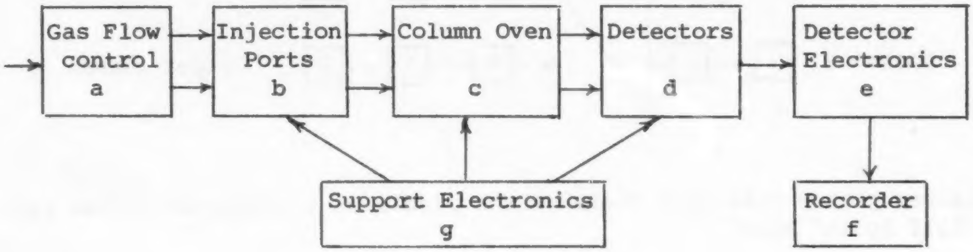
Bracket Placement
e.g., no flow: Rotameter indicates flow,

(e) Pause and think - is there only one malfunction? What was changed or done recently that could cause the problem? Check with other personnel using the instrument.

(f) Verify by alternate tests.

(g) Speed, availability, and complexity determine replacement or repair procedure.

9 D GAS CHROMATOGRAPH SERVICE BLOCK DIAGRAM


Gas Flow control a
Injection Ports b
Column Oven c
Detectors d
Detector Electronics e
Support Electronics g
Recorder f

(a) Gas flow control: Purifies and dries the carrier gas, splits carrier gases to the columns and detectors. Controls and regulates the gas flow.

(b) Injection block: Vaporizes the sample, introduces the sample into the carrier gas stream.

(c) Column oven: Houses columns, provides a dynamic constant or programmable temperature environment.

(d) Detectors: Equalize gas temperature, detection of gas stream components, exhaust gases.

(e) Electrometer: Conditions the detector signal and attenuates the output for transmission to the recorder.

(f) Recorder: Displays an analog signal (chromatogram).

(g) Support electronics: Controls the temperature of the injection ports, column oven, detectors; indicates temperatures, indicates control voltage.

9 E GAS INLET SYSTEM OF THE GAS CHROMATOGRAPH

Components:

(a) Carrier gas cylinder

(b) Two stage gas regulator

(c) Molecular sieve drying trap

(d) Copper tubing to the instrument and rotameters

(e) Rotameters

(f) Differential pressure flow controllers

(g) Tubing to the inlet

(h) Inlet/transfer block

(1) Thermocouple

(2) Resistance thermometer

(3) Injection port: septum nut, septum, septum washer, insert retainer, glass demister trap.

Comments:

(a) The carrier gas cylinder used in gas chromatography is generally size "A" dry pumped nitrogen. When the EC detector is operated in the "pulsed mode", the carrier gas is then 5 or 10 percent methane in argon. The carrier gas must be dry and contain less than 5 ppm O_2. Contamination of the carrier will severely affect performance.

(b) A two stage gas regulator should be used to reduce and regulate the carrier gas pressure. The first stage gauge indicates the cylinder pressure while the second stage indicates the reduced pressure to the chromatograph. A diaphragm valve on the regulator allows control of the output pressure, which should be 40-50 psi.

(c) Molecular sieve drying traps should be installed between the regulator and the chromatograph to prevent water and hydrocarbon contamination from entering the gas chromatographic flow system. Molecular sieve 13X (1/16 inch) pellets have been found satisfactory for the filter load and should be baked-out at 350°C in a nitrogen stream for four hours prior to use and capped off for storage. It is advisable to do this with every cylinder change. Occasionally the dryer body should the cleaned with hexane/acetone before reloading. It may also be necessary to flame the dryer frit to expel all contamination.

(d) Copper tubing, 1/8 inch instrument grade is used between the filter dryer and the instrument. This tubing should be rinsed with CH_2Cl_2 and then acetone before installation. If the old tubing is used, it should be also flamed. It is also advisable to clean all Swagelok fittings before installation. Swagelok nuts should be placed on the tubing for use before being placed on the instrument. This insures proper Swage connection and reduces the possibility of damage during installation.

(e) The rotameters generally used in the carrier stream are the Brooks "Sho Rate" 150. They are calibrated for the pressures encountered in GC work. Charts of the calibration curves are readily available. Contamination of the rotameters may cause the float to stick. Moisture in the tube will appear as a ring around the float. The tube must be removed by loosening the hex screw at the top of the rotameter body. Do not attempt to clean the tube in the rotameter body since solvents will attack the "O" rings or seals causing them to swell. It is also possible that the "O" rings will absorb the solvent and bleed vapor into the system. The tube should be cleaned with hexane, acetone, and Freon MS-180. A flow meter should not be placed downstream of the flow controller but rather between the carrier gas inlet and the flow controller.

(f) The differential flow controllers (Brooks) are composed of a needle valve/seat assembly and a diaphragm, preferably Teflon, to maintain a constant flow of carrier through the column, even though the pressure drop across the column changes. The controller requires at least 25 psi for proper operation and may flutter under lower pressures. It is recommended that 40-50 psi be used to operate them properly. In programming, it is recommended that 60 psi be used to insure proper response of the system.

The needle valve/seat assembly may occasionally stick. This occurs when the needle becomes lodged in the seat. The valve must be taken apart and the needle and seat cleaned. Experience has indicated that when this occurs, a new needle valve/seat assembly is advised. The main cause of damage to the assembly is through improper operation: never close down forcibly, never open wide past the point that the float rests in the upper part of the flow tube. Since these are differential controllers, there will still be slight gas flow when the rotameter float is at zero. This is not uncommon as a flow controller is not a shut-off valve!

It is advisable to install a small frit spring-loaded filter on the outlet of the flow controller. This is a Brooks type 8501/8502 unit that will protect the controller from flashback and trap solid contaminants from the carrier gas, or filter.

(g) Copper tubing 1/8 inch od is used from the flow controller and is joined to the 1/8 inch od stainless steel tubing of the inlet assembly with 1/8 Swage to 1/8 Swage unions. It is advisable to replace these unions with a Swagelok slide valve, 1/8 inch. This will aid in checking the system for leaks up to this point and may enable operation of one column while repairs are being carried out. By closing off the slide valve, the float should drop to zero; if it does not, there is a leak up to that point. The 1/8 inch stainless steel line is welded into the inlet, which is secured by screws into the inlet heater block.

(h) The inlet block is an aluminum block with facility for inserting the inlet thermocouple and a heater cartridge. There is also an orifice for inserting a resistance thermometer. With the addition of other detectors in the head compartment, this resistance thermometer may not be used in many cases and the inlet is heated (225-230°C) by controlling voltage through a Variac voltage regulator. This inlet tube assembly has a septum retaining nut which holds the septum in place and has a small orifice for insertion of the syringe needle into the port. The septum washer (stainless steel) is placed under the septum and above the insert retainer to allow for a flat surface above and below the septum. The glass insert retainer should be turned down until it comes in contact with the glass insert and then backed off 1/8 turn. The Vykor glass insert should be removed daily and replaced with a clean one while the used insert is cleaned in the prescribed manner (Subsection 5.J. in Section 5).

Care should be taken to insure that the thermocouple and heater leads do not become frayed. A small amount of insulating material or glass tape should be used between metal surfaces and these wires. Avoid sharp bends close to the element.

9 F PROCEDURE FOR ISOLATING PROBLEMS IN FLOW SYSTEMS OF ELECTRON CAPTURE EQUIPPED GAS CHROMATOGRAPHS

(a) Allow the column oven to cool to ambient temperature, set all rotameters to zero, and close off all oven exit ports with 1/4 inch Swagelok plugs.

(1) If the EC detector is suspect because of poor total response regardless of the amount of polarizing voltage applied to the detector, install an EC detector with a new foil.

(2) Prepare a line filter filled with 13X (1/16 inch) molecular sieve pellets and activated as previously described. Place it at the dual-stage regulator output of the carrier gas tank. The dual-stage regulator should be set to deliver 40 psi to the system.

(3) Insure that a cylinder of carrier gas which is known to be free of contaminants is being used in the system.

(4) Attach the detector purge line to the proper purge connection and set the rotameter for a flow of approximately 90 ml/min.

(5) Adjust the detector temp-set controller to maintain a detector temperature of approximately 200°C. If a faulty temp-set is encountered, substitute a Superior type B variac.

(6) Obtain a profile for the background current signal (BGS) by increasing the polarizing voltage in five (5) volt increments, noting on the chart the step characteristics and the maximum deflection voltage.

(7) The resulting profile should approximate the profile provided with the detector in characteristics and maximum deflection voltage although slight variations may occur. Particular attention should be paid to the step increase from 0-5 volts and from 5-10 volts. A leak or contaminant in the flow system are common causes of a poor profile (Figure 5-E in Section 5).

(b) Corrective measures for locating faults in the purge loop.

(1) Check for leaks at all fittings in the purge loop with "Snoop", beginning at the cylinder. At this point, also use "Snoop" to check for leaks on the valve control stems.

(2) Change the detector purge line to another rotameter on the purge module.

(3) Clean all lines in the purge loop or replace with new ones which have been cleaned and flamed. All tubing that is changed should be replaced with instrument grade tubing, and it is recommended that this tubing also be cleaned prior to installation.

(4) If the condition of the carrier gas was not known at the time the above tests were made, a new cylinder of carrier gas should be tested. All new cylinders should be checked for leaks at their welds and outlet valves.

(c) After obtaining a satisfactory profile with purge, place 1/4 inch Swagelok plugs on all four column inlet fittings inside the oven.

(1) Replace or remove the glass demisters in the inlets.

(2) Replace the inlet septums with new and preconditioned septums.

(3) Adjust the column #1 flowmeter to deliver approximately 90 ml/min of carrier gas. The rotameter float should rise and very shortly should drop to zero if there are no leaks in the column #1 flow system. Repeat this on the other carrier systems. If none of the floats fall to zero, the leak is usually common to all ports. The most common area for this type of leak is at the "pigtail" fitting on the instrument rear although there have been instances of such leaks occurring due to a cracked inlet block.

(d) Corrective measures for locating leaks in the carrier flow system.

(1) Open the 1/8 to 1/8 Swagelok unions on the rear of the chromatograph where the copper lines meet the stainless steel lines and cap off the copper line at this point. Upon applying carrier flow as stated in (c) (3), the float should drop to zero. If the float does not fall, the leak is in the flow controller or rotameter. It is possible to tighten slightly the flow tube in the rotameter by the hex adjustment located on the top of the rotameter housing.

(2) "Snoop" all lines from the "pigtail" to the Swagelok plug and observe for leakage.

(3) Observe the rotameter float for "bounce" or rapid but slight fluctuations; this will usually indicate a faulty diaphragm. Do not attempt to repair the diaphragm but replace the total unit with a new flow controller.

(e) Install in all ports empty glass columns that have been thoroughly cleaned, taking care that they are properly seated.

(1) Allow the system to remain under carrier flow for at least 30 minutes to evacuate air introduced into the loop during installation of the columns.

(2) Follow the procedures outlined in (a) (6) and (a) (7) for each port.

(3) If an acceptable BGS profile is obtained, the flow system is free of leaks or contamination under ambient temperatures.

(4) Slowly increase the temperature of the inlet, oven, and transfer lines to their operating levels and obtain a BGS profile for all ports.

(f) Corrective measures for locating faults in the carrier flow loop.

(1) Test for septum leaks if new septums were not used by rapidly cooling down the septum nut with water and allowing a small quantity to remain in the septum nut depression. Observe this water-filled depression for bubbles that would indicate a leak.

(2) Insure that the columns are seated and sealed properly. It is usually advisable to tighten the columns an additional 1/4 turn after heat has been applied to the oven.

(3) Tighten the transfer line fittings, but take care not to strip the fitting threads.

(4) Inspect for a cracked block. By turning up the carrier flow to a high level, it is sometimes possible to hear the escaping gas.

(5) If the steps taken do not correct the problem, it may be assumed that there is contamination in the carrier loop.

i. Remove all fittings in the flow loop and clean or replace them.

ii. Clean the inlet and outlet ports while heated and under carrier flow with Pre-Post 1001 cleaner. Use a pistol cleaning brush.

iii. Flame all lines under carrier flow where possible.

iv. Remove and clean the flow controllers and dry thoroughly with carrier gas. Be sure to remove all moisture from the controller diaphragms.

v. Add an in-line filter loaded with 13X molecular sieve at the junction of the copper and stainless steel tubing until an acceptable BGS is obtained. If, after the addition of the in-line filters, a proper BGS is obtained, it may be assumed that the problem is in the rotameter or flow controller area. Do not operate with these filters permanently. Install a new carrier module and replace all tubing from the bulkhead fittings to the in-line filters.

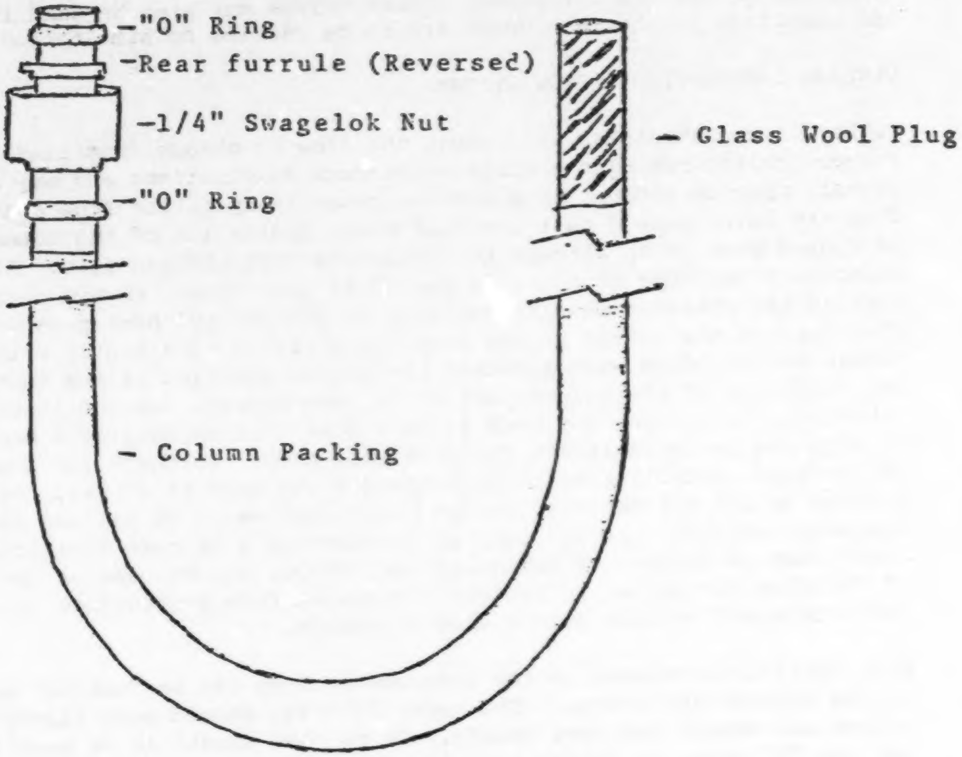
vi. Install well conditioned columns and allow the system to equilibrate. If in-line filters remain in the carrier loop, allow additional equilibration time because of the greater volume in the loop. Flow control changes will take approximately 30 minutes to equilibrate with these filters in the system. Obtain a BGS profile from all ports. Insure that the columns are not filled to the point where the packing will come in contact with the metal inlet and outlet fittings. The higher temperature at these points may cause the column packing to bleed. Slowly raise the oven temperature to 100°C and obtain a BGS profile. If it is satisfactory, raise the oven temperature to full operating temperature and obtain a BGS profile. If it is again satisfactory, the instrument should now be in full operating condition.

It has been the experience of the RTP Laboratory that in-line filters loaded with 13X molecular sieve are superior in performance to those loaded with type 5A. It is therefore recommended that all filters used be charged with 13X and conditioned as prescribed. The addition of in-line filters at the rear of the instrument between rotameters and column inlets is not meant to be a permanent change. The installation of these filters allows operation of the instrument until it is convenient to obtain materials to make repairs. As an additional means of rapidly checking the system for leaks, it is suggested that the unions on the instrument rear where the copper tubing meets the stainless steel tubing be replaced with Swagelok slide valves (#200-1/8 SV-6). These valves will enable the chromatographer to totally shut off any individual carrier loop so that the float in the rotameter, flow controller, and lines can be checked for leaks. These valves may also be used if repairs and operation of the instrument are to be carried on simultaneously.

GENERAL INFORMATION - FLOW SYSTEM

Faulty flow controllers will cause the flow to change from time to time. The controller may also exhibit short term fluctuations and may completely open or close. Care must be taken in operating flow controllers; they are never opened past the full scale indication of the rotameter or closed down in an attempt to completely shut off gas flow. Always maintain a cylinder pressure in the 40-50 psi range. Always change the carrier gas cylinder when it depletes to 500 psi cylinder pressure. Check flow through the system at the detector effluent line weekly with a bubble meter. This will indicate the proper function of the instrument and condition of the column packing to some degree. As the instrument vibrates, the columns may tend to pack down tighter causing a decrease in flow and may also affect the retention time. To check for a worn or bad flow controller diaphragm, operate the unit at 40 psi, noting a setting on the rotameter. Increase the pressure to 50 psi and note the rotameter setting. If it shows an increase of 4 or more divisions the controller is faulty. A Brooks filter, #8501, may be used at the outlet of the flow controller to protect the system from particulate contamination and to some extent from flashback.

When installing columns in the instrument, they can be "set-up" as shown in the diagram following. The upper "0" ring should seat firmly on the column and should not move freely. In no case should it be necessary to use two "0" rings to obtain a seal. The rear furrule is installed in a reversed procedure than would be used in a metal to metal fitting installation. The lower "0" ring is not critical and is used simply to support the Swagelok nut. A clearance of at least 1/8 inch is recommended between the column packing and the fittings after installation is completed.



9 G TEMPERATURE CONTROL AND INDICATION IN THE GAS CHROMATOGRAPH

The inlet and transfer system may be controlled by a feedback bridge type SCR controller. This controller uses a resistance thermometer and a potentiometer for control. It has been found advantageous to utilize variac control of the inlet and transfer where possible (cost- and trouble-free operation). Variac control is possible when ambient conditions are stable.

The electron capture detector temperature should always be controlled by the feedback bridge type SCR controller as minute changes here may cause cycling or shift due to small changes in ambient temperature or improper insulation in the detector cage. There should be no detector body area exposed, as slight air currents may cause cycling or drifting baselines.

A pyrometer (0-500°C) is generally used to indicate the temperature of various thermocouples in the instrument. A switch located adjacent to the pyrometer is used to connect various thermocouples into the circuit to be monitored. The thermocouples used in the chromatograph are usually terminated in a compensator which is a cold junction reference bridge circuit that compensates for ambient room temperature. This circuit is dependent upon thermistors in the bridge circuit and mercury (RM-12) batteries that should be checked monthly with a battery checker or whenever temperature indications appear faulty. (Usually one thermocouple is placed in the open air to rough-check the pyrometer against ambient temperature). Always be sure that battery contacts are clean.

Oven temperature and control is obtained by:

(a) Two resistance wire heaters in the oven walls secured to plates attached to the walls and wired in series with a limit switch.

(b) Two thermocouples (metal sheathed). T/C is used with older type programmer units. Ribbon Resistance Thermometers (50 ohms) will be used in newer types for temperature sensing.*

(c) Fan motor and squirrel cage blower assembly.

(d) Damper system.

The oven is generally a stainless steel unit insulated with micro fiber insulation cover. The design permits rapid heating and cooling dynamically to desired temperature equilibration.

The top sheathed thermocouple is used as the temperature indicating unit and the bottom sheathed thermocouple* is used for programmer control. This placement is non-critical.

The programmer contains an initial control circuit which is used mainly in isothermal control of the oven and a final control circuit activated for programming. The programmer circuitry may be used to raise the temperature at a specific rate and automatically return to a set temperature by proper use of the adjustments on the front panel. The oven temperature controller will not be activated until the fan/blower switch is turned on.

The onset of pyrometer problems is often insidious, and problems may be prevalent for some time before the operator becomes aware of them. One simple and inexpensive means of monitoring this, if the oven design will permit, is to drill a 1/8 inch hole in the oven door at a point opposite the center of the columns; insert through the hole the 8 inch stem of a dial face bimetallic thermometer (Weston model 4200, 0-250°C). While these thermometers are not highly precise, they are sufficiently accurate to provide the operator with an indication of trouble in the pyrometer network. If the dial face thermometer is reading 185°C and the pyrometer readout is 200°C, a problem is indicated not only in the column oven temperature but in the other heating modules in the instrument, i.e., injection port, detector, transfer line, etc.

9 H DETECTOR AND ELECTROMETER

The detector and electrometer are integral parts of the gas chromatograph. They are connected together by BNC to BNC Teflon coaxial cables, one for the polarizing voltage and the other for the detector signal. The electrometer supplies a negative DC voltage to the detector at a regulated constant rate. The radioactive detector source is encased in a Teflon (^{3}H) or ceramic (^{63}Ni) cylinder. This cylinder, in turn, is encased in a stainless steel block which serves as a heat sink heated by a 50 or 100 watt heat cartridge. When a ^{3}H (tritium) detector is used, an adjustable limit switch in series with the heat cartridge prevents the detector from being heated above 225°C. If the temperature is allowed to exceed 225°C, excessive losses of tritium from the foil or damage to the Teflon parts will result. Such temperatures create no problems with the ^{63}Ni detector, thus a limit switch is not needed.

The electrometer input attenuator is comprised of high resistance glass resistors forming a decade stepping switch. These resistors are affected by dust, temperature, and light. They must be maintained extremely clean and never hand touched. The highest attenuation available is 5 x 10^{-13} amps. This is obtained at the 0.1 setting. A minimum attenuation of 5 x 10^{-8} amps is obtained at the 10^{6} position.

The output attenuator is a binary resistance switch that enables further attenuation in a 1 to 256 range. A potentiometer in the output section of the unit adjusts zero balance of the electrometer amplifier to permit adjustment of the output voltage for zeroing the strip chart recorder. A bucking control utilizing two glass resistors for normal or high bucking is located on the electrometer rear apron. This bucking control is the coarse bucking allowing either 10^{-8} for electron capture operation or 10^{-10} for flame. A 10 turn potentiometer is located on the front panel for fine bucking control.

Internally, the electrometer contains two plug-in printed circuit boards, one for power conditioning and the other for amplification, zero, and balance. This is in addition to the input and output attenuators. It is important (due to the extreme sensitivity) to operate the electrometer with its cover correctly in place (otherwise noise will be excessive due to stray field pick up).

Electrometer Problems:

(a) Can't zero and/or can't buck out - possible cure:

(1) Check zero and bucking pot on front panel for continuity.

(2) Check 1 percent resistors on amplifier board for continuity.

(3) Check 2N699 transistor on amplifier board for leakage.

(4) Check 4 mfd @ 50V non-polarity capacitor on amplifier board.

(5) Check all zener diodes on power supply board.

(b) To adjust trim pots on amplifier board:

With output attenuation on 1, turn input attenuator to off and turn zero pot on front panel five turns from either extreme. Then adjust zero balance trim pot until recorder reaches zero.

Electrometer Drift Check:

(a) Set master switch to off and completely disconnect the electrometer from all test equipment.

(b) Connect recorder signal cable to the electrometer.

(c) Set input attenuator to 0.1 and output attenuator to 1.

(d) Adjust bucking potentiometer for recorder indication of 50 divisions.

(e) Set recorder to slow speed.

(f) Allow units to run in this condition until recorder does not deviate more than 5 divisions per hour.

(g) Set output attenuator from position 1 to position 256 slowly, noting pen deflection. This should not exceed 0.25 chart division through the total range.

Solid State Linearizer:

A recent innovation introduced for use with electron capture detector systems in the "constant current pulsed mode" is a solid state linearizer. The linearizer enables the chromatographer to operate the electron capture detector over a dynamic range of at least 20,000:1.

The linearizer requires a warm-up period of at least 12 hours after installation or after any flow interruption. Argon-5% methane is the preferred carrier gas; however, a 10,000:1 dynamic range may be obtained using high purity nitrogen.

Malfunctioning of the solid state linearizers has been observed where the recorder will suddenly go off scale and remain so until the unit is shut down for a period of time. Upon reactivation of the system, the unit appears to function normally. If this occurs, refer to the operational manual schematics and:

(1) Change R_2 and R_3 to 1K½W
(2) Change VR_2 to 7.5 or 8.2V
(3) Add 330 mfd at 10 VNP across R14
(4) Add 1 mfd at 50V in series with a 10 ohm ½W across CR5.

These changes will improve the operation of the linearizer and reduce noise to an acceptable level. It may be necessary to re-zero the unit after these changes. The Tracor service manual procedure "2" states "Remove clip lead." This is incorrect. The clip lead should be retained. In procedure "3", do not clip a shunt lead from E_4 to ground and do not adjust to zero but to 30 MV.

Printed circuit board 1700 - 504400H cannot be repaired or aligned in the field and must be returned to the factory for replacement.

All printed circuit boards in a linearizer should be removed annually and spray cleaned with Freon MS-180.

9 I OBSERVATION OF PROBLEMS ON CHROMATOGRAMS

(a) Peaks return below baseline: dirty or partially depleted detector foil - clean or replace foil (See Figure 5-5, A in Section 5).

(b) Peaks have flat tops: check for proper oven and detector temperature and recorder gain control.

(c) Insufficient peak height: check for proper attenuation settings, proper amount of injection, recorder response.

(d) Tailing peak: check for proper operating temperature and gas flow.

(e) Stepping baseline (may be observed on peaks): check for dirty recorder slide wire, line voltage changes, recorder drive, recorder gain control.

(f) Noise level: check for approximately 1 division of noise @ 10 x 8 attenuation.

(g) Spikes: check external polarizing voltage unit, line noise, noisy temperature set controller, dirty system, regulation diaphragm.

(h) Rapid cycling: check oven temperature programmer, oven control thermocouple, compensation circuit, temperature set controllers, limit switches.

(i) Excessive noise in baseline: check for module noise by elimination on substitute, ground loops, recorder gain (properly set?), cable connections (coaxial), leaks in flow system.

(j) Noise with erratic spikes: check for proper carrier, clean carrier, leaks, ground loops, column bleed.

(k) Slow cycling baseline: check oven limit switch, damper operation, control thermocouple, thermocouple compensator.

9 J DETECTOR BGS RESPONSE

(a) Normal detector response - has good maximum signal (BGS).

(b) Abnormal detector response - has poor stepping, does not saturate at approximately 25 volts (Figure 5-E, Section 5).

Possible Problems:

(1) Moderately contaminated carrier gas.

(2) Bleeding or unconditioned column (absorbs BGS).

(3) Positive voltage on detector.

(4) Leak in system.

(5) Detector in heating cycle (wait until pyrometer stabilizes).

(6) Reversed coaxial leads from electrometer to detector.

(7) Contaminated radioactive source in detector.

(8) Contaminant flowing from previous injections (residue bleed).

(9) Dirty, bleeding, torn septum.

Troubleshooting:

(1) Leave column cold. Eliminates (2) and (8).

(2) Check system from tank to detector fittings. Eliminates (4).

(3) Observe voltage with VOM. Eliminates (3).

(4) Check coaxial connectors. Eliminates (6).

(5) Check detector temperature for stability. Eliminates (5).

(6) Observe standard solution injection - if peaks are not below base, eliminates (7).

(7) Replace septums with conditioned ones. Eliminates (9).

(8) Change carrier gas tank. Eliminates (1).

9 K TROUBLESHOOTING THE MICROCOULOMETRIC SYSTEM

(a) No flow from pyrolysis tube.
Symptom: no flow indicated on bubble meter.

Probable causes:

(1) Empty gas tank.

(2) Pressure not on.

(3) Broken pyrolysis tube.

(4) Broken column.

(5) Broken flow meter.

(6) Broken flow control.

Troubleshooting procedure:

(1) Check tank pressure.

(2) Set proper output pressure from tank.

(3) Remove and check pyrolysis tube.

(4) Remove and check column.

(5) Remove and check flow meter.

(6) Check output from flow control.

(b) Furnace will not heat.
Symptom: no temperature increase on pyrometer.

Probable cause:

(1) Heat control not on.

(2) Thermocouple open.

(3) Furnace heater open.

(4) Heat control defective.

Troubleshooting procedure:

(1) Turn heater control on.

(2) Check to see if furnace is red on inside, check thermocouple with ohm meter.

(3) Check furnace heater resistance with ohm meter.

[1](4) Check 0-110 VAC output from heat control.

(c) Inlet block will not heat.
Symptom: no temperature increase on pyrometer.

Probable cause:

(1) Heat control not on.

(2) Thermocouple open.

(3) Furnace heater open.

(4) Heat control defective.

Troubleshooting procedure:

(1) Turn heater control on.

(2) Feel block, check thermocouple with ohm meter.

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[1] Caution: use only volt-ohmeter. Do not use vacuum volt meter.

(3) Check resistance of block heater with ohm meter.

[1/] (4) Check 0-110 VAC output from heat control.

(d) No output from A-100 test box.
Symptom: will not read 1 VDC on volt meter.

Probable cause:

(1) Duo-Dial not set at 1000.

(2) Batteries defective.

(3) A-100 defective.

Troubleshooting procedure:

(1) Set Duo-Dial at 1000 (10 turns).

(2) Check batteries 1.35 VDC. Replace if necessary.

(3) Substitute A-100.

(e) Recorder will not zero.
Symptom: microcoulometer output range at 000, recorder will not zero.

Probable cause:

(1) Cable not connected from coulometer to recorder.

(2) Coulometer batteries defective.

(3) Coulometer defective.

(4) Recorder defective.

Troubleshooting procedure:

(1) Connect cable from coulometer to recorder.

(2) Check batteries 1.35 VDC. Replace if necessary.

(3) Substitute coulometer.

(4) Substitute recorder.

[1/] Caution: use only volt-ohmeter. Do not use vacuum volt meter.

(f) No bias output
Symptom: meter does not move when bias control is adjusted.

Probable cause:

(1) Function switch not in proper position.

(2) Batteries defective.

(3) Coulometer defective.

Troubleshooting procedure:

(1) Set function switch to Bias Read.

(2) Check batteries 1.35 VDC. Replace if necessary.

(3) Substitute coulometer.

(g) Coulometer will not balance in Generator Read.
Symptom: meter is either extreme left or right.

Probable cause:

(1) A-100 not connected.

(2) Batteries defective.

(3) Coulometer defective.

(4) A-100 defective.

Troubleshooting procedure:

(1) Connect A-100 to coulometer.

(2) Check batteries 1.35 VDC. Replace if necessary.

(3) Substitute coulometer.

(4) Substitute A-100.

(h) Baseline noisy when connected to A-100 test box, Operate mode.
Symptom: recorder baseline 1-1 1/2 percent noise at range 200/Lo Gain, range 50/Hi Gain.

Probable cause:

(1) Ground not connected from A-100 to coulometer.

(2) A-100 defective.

(3) Coulometer defective.

(4) Recorder defective.

Troubleshooting procedure:

(1) Connect ground from A-100 to coulometer.

(2) Substitute A-100.

(3) Substitute coulometer.

(4) Substitute recorder.

(i) Unable to balance coulometer, cell connected.
Symptom: meter extreme left or right.

Probable cause:

(1) Function switch in wrong position.

(2) Batteries defective.

(3) Cell needs flushing.

(4) Cell defective.

(5) Coulometer defective.

Troubleshooting procedure:

(1) Place function switch in Operate position.

(2) Check batteries 1.35 VDC. Replace if necessary.

(3) Flush cell.

(4) Substitute cell.

(5) Substitute coulometer.

(j) Recorder baseline noisy, cell connected.
Symptom: baseline 1 1/2 - 2 percent noise, range 200/Lo Gain, range 50/Hi Gain.

Probable cause:

(1) Cell contaminated.

(2) Gas contaminated.

(3) Coulometer defective.

(4) Recorder defective.

(5) Flow system has leak.

Troubleshooting procedure:

(1) Clean cell.

(2) Open vent, substitute gas.

(3) Substitute coulometer.

(4) Substitute recorder.

(5) Test system for leaks.

(k) Poor sensitivity.
Symptom: standard injected produces little or no response.

Probable cause:

(1) Wrong standard.

(2) Wrong attenuation on coulometer.

(3) Cell needs replating.

(4) Pyrolysis tube contaminated.

(5) Leak in flow system.

Troubleshooting procedure:

(1) Check standard.

(2) Check range and gain setting.

(3) Replate cell.

(4) Replace pyrolysis tube.

(5) Test flow system for leaks.

9 L TROUBLESHOOTING COULSON ELECTROLYTIC CONDUCTIVITY SYSTEM

(a) High background.
Symptom: unable to zero system with bridge. Bridge attenuator x8 or below.

Probable cause:

(1) Recorder zero inaccurate.

(2) Water contaminated.

(3) Ion exchange capacity exhausted.

(4) Gas contaminated.

(5) Bleeding column.

(6) Leak in gas system.

Troubleshooting procedure:

(1) Attenuator at short, zero recorder.

(2) Change water.

(3) Change ion exchange bed.

(4) Check background with the cell disconnected from furnace, change gas.

(5) Replace column with glass jumper.

(6) Test system for leaks.

(b) Low sensitivity.
Symptom: noticeable loss from previous response.

Probable cause:

(1) Leak in flow system.

(2) Contaminated pyrolysis tube.

(3) Contaminated Teflon transfer line.

Troubleshooting procedure:

(1) Test system for leaks.

(2) Replace or re-cure pyrolysis tube.

(3) Replace Teflon transfer line.

(c) Noisy baseline.
Symptom: baseline noisy, 3 percent or more at x2 on attenuator.

Probable cause:

(1) Recorder gain too high.

(2) Bridge not properly grounded.

(3) Ion exchange capacity exhausted.

(4) Bridge defective.

(5) Dirty cell.

(6) Improperly cured column.

Troubleshooting procedure:

(1) Set bridge attenuator to x2. Adjust recorder gain.

(2) Connect a jumper from bridge white terminal to recorder ground.

(3) Change ion exchange bed.

(4) Substitute bridge.

(5) Clean cell with 10 percent solution of HF, rinse with distilled water.

(6) Recondition column.

(d) Loss of gas flow.
Symptom: bubbles not present in cell.

Probable cause:

(1) Gas tank empty.

(2) Broken pyrolysis tube.

(3) Broken column.

(4) Valve blocked.

(5) Broken flow control.

Troubleshooting procedure:

(1) Check tank pressure.

(2) Remove and inspect pyrolysis tube.

(3) Remove and inspect column.

(4) Check flow through valve and clean if necessary.

(5) Check output from flow control.

(e) Loss of furnace heat.
Symptom: pyrometer does not read 820°C or the set temperature.

Probable cause:

(1) Heat control off.

(2) Thermocouple open.

(3) Furnace heater open.

(4) Heat control defective.

Troubleshooting procedure:

(1) Push heat control knob to turn heat on.

(2) Visually check furnace. Insure it is red on inside, check thermocouple with ohm meter.

(3) Check resistance of furnace heater with an ohm meter.

[1] (4) Check variable 1-110 VAC output of heater control with volt meter.

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[1] Caution: use only a volt-ohmeter. Do not use a vacuum tube volt meter.

(f) Loss of inlet block heat.

Probable cause:

(1) Heat control off.

(2) Thermocouple open.

(3) Block heater open.

(4) Heat control defective.

Troubleshooting procedure:

(1) Push heat control knob to turn heat on.

(2) Check thermocouple with an ohm meter.

(3) Check resistance of block heater with an ohm meter.

[1](4) Check variable 0-110 VAC output of heater control with volt meter.

9 M TROUBLESHOOTING THE FLAME PHOTOMETRIC DETECTOR (FPD)

(a) Noisy baseline[2]

Probable cause:

(1) Detector temperature too high.

(2) 750 volt power supply noisy.

(3) Noisy electrometer.

(4) Damaged photomultiplier tube.

(5) GC column bleeding.

Troubleshooting procedure:

(1) Lower temperature to 160-170°C.

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[1] See footenote on page 28.

[2] In any case of noisy baseline, make certain the recorder gain is properly adjusted and the slidewire is clean.

(2) Disconnect cable from end of PM tube and observe baseline. If noise continues, 750 volt power supply may be the cause. Repair or replace.

(3) Continuing noise if cable from back of electrometer is disconnected indicates bad electrometer.

(4) Continuing noise with extinguished flame indicates damaged PM tube if electrometer and power supply have checked good. Replace tube.

(5) Recondition or replace column.

(b) Low sensitivity.

Probable cause:

(1) Dirty filter.

(2) Dirty window.

(3) Damaged photomultiplier; low sensitivity will probably be accompanied by excessive noise.

(4) Improper polarizing voltage.

(5) Light leaks.

(6) Improper flow rates.

(7) Loose cables.

Troubleshooting procedure:

(1) Remove filter and clean with lens tissue. Be sure to turn off polarizing voltage before removing PM tube.

(2) Remove PM tube and filter and look into back of detector. Replace window if coated with grey deposit.

(3) Replace PM tube.

(4) Perform voltage/injection profile.

(5) A shift in baseline will occur by shading the detector burner housing.

(6) Insure all flow rates are correctly set.

(7) Check that all cables are tight.

9 N EPILOG

The reader is cautioned against leaping to a conclusion that the source of an operating problem is an instrumental malfunction when in fact it may be something else entirely. For example, if the operator makes a series of injections of a relatively "dirty" extract for electron capture detection (such as the 15 percent ethyl ether-petroleum ether cleanup fraction of fat), certain symptoms may appear on the chromatogram which could suggest electronic problems. Peak height response may be greatly depressed, and significant peak tailing may occur. However, these manifestations are simply electronic symptoms and not causes. The cause of the problem in this case would be overloading and contamination of the GC column (see Figure 4-1 in Section 4). Because of the visible similarities on the chromatograms of electronic *vs.* other problem sources, the operator should not proceed post haste to tear the instrument apart without first checking out the possibility of other problem sources. In general, if the instrument has been performing satisfactorily up to the time of starting chromatography on a new series of samples, it would seem probable that the problem may reside in the sample extract rather than in the gas chromatograph.

Section 10

TRAINING OF PESTICIDE ANALYTICAL CHEMISTS

This chapter is by far the shortest in the manual, and the reader may question the logic of devoting a special section to this subject. During the years of operating the interlaboratory quality control program described in Section 2, the editors observed overwhelming evidence that participating laboratories with chemists who had formal, specialized training demonstrated far superior analytical performances than did those laboratories which lacked this advantage. We, therefore, regard training as a highly important subject and deserving of special treatment.

Although many good programs are available in undergraduate and graduate schools for the training of analytical chemists, few, if any, specifically train pesticide analysts. An undergraduate or graduate student on a research project with a professor interested in development of residue analytical methods does receive valuable training and experience, but such professors are few and far between in American education institutions. A number of companies in the private sector offer short courses particularly designed for training users of company-produced equipment. A certain few universities and private educational organizations run short courses touching upon a few of the highlights of pesticide residue analysis. Some governmental agencies operate similar short training courses.

The residue chemist must not only be familiar with the techniques of trace analysis in general and of residue analysis in particular, but he must be able to perform routine service and adjustments and preventative maintenance, such as module replacements and replumbing, on his instruments. In order to achieve these abilities, a generally trained analytical chemist should be given on-the-job training by an experienced residue chemist when he is hired, if at all possible. Since this is often not possible, especially in smaller laboratories, this Manual is designed to substitute, in small part, for such training and to help the analyst recognize certain pitfalls and to better perform analyses of biological and environmental media. There is, however, no really satisfactory substitute for intensive, practical bench training of the type formerly provided by the EPA Perrine Primate Laboratory Training Program, Perrine, Florida. During the years of conducting the interlaboratory quality control program described in Section 2, it was very apparent that those laboratories which took most advantage of the Perrine training facility recorded far better analytical performances on round robin samples than laboratories not participating in the training program. As a specific illustration of this, the reader is referred to Table 10-1 (copied from Table 2-7 in Section 2) which lists the relative performance ranking of 34 laboratories in one interlaboratory check sample exercise.

The top eight laboratories, performancewise, had previously sent personnel to the Perrine training program. Of the 17 laboratories in the top half of the table, 10 of these laboratories had Perrine-trained chemists. Of the 17 laboratories in the lower quality half of the table, only one laboratory near the top of the lower half had sent personnel for training. All laboratories which had Perrine-trained personnel are check-marked next to their identifying code numbers.

The editors feel that the data shown by this table provides most conclusive evidence of the value of a proper training program in the potential quality output of a pesticide analytical chemist. Unfortunately, however, the agency saw fit to discontinue the Perrine training program, the only one of its kind in existence, but it is hoped that some educational institutions or governmental agency will recognize the need and set up programs to provide such training, and that laboratory supervisors will take advantage of these in urging their residue chemists to obtain and refresh, on a continuing basis, their training and knowledge in analytical and instrumental areas. Rapid developments in instrumentation and new techniques dictate a constant need for training and retraining in a field as highly complex as that of residue analytical chemistry. Furthermore, recent disclosures of pollution of the nation's air and water by a wide variety of organic compounds including pesticides point up the need for scientists with a sound background of analytical expertise.

TABLE 10-1

RELATIVE PERFORMANCE RANKINGS

CHECK SAMPLE NO. 26, MIXTURE IN SOLVENT

Lab. Code Number	Compounds Missed	False Identifications	No. of Rejects 1/	Total Score 2/
✓ 161.	0	0	0	198
✓ 137.	0	0	0	198
✓ 135.	0	0	0	197
✓ 162.	0	0	0	197
✓ 87.	0	0	0	197
✓ 113A.	0	0	0	197
✓ 113.	0	0	0	196
✓ 85.	0	0	0	196
48.	0	0	0	195
130.	0	0	0	195
✓ 66.	0	0	0	195
73.	0	0	0	194
✓ 72.	0	0	0	194
84.	0	0	0	192
89.	0	0	0	192
88.	0	0	1	189
83.	0	0	0	189
96.	0	0	2	187
97.	0	1	0	181
164.	0	1	4	169
✓ 68.	1	0	1	168
92.	1	0	0	168
93.	0	1	2	164
90.	1	0	2	159
53.	1	0	1	158
163.	1	1	0	157
95.	2	0	0	146
160	2	1	0	133
45.	0	0	6	128
71.	3	0	3	127
52.	2	1	2	123
47.	3	0	1	115
69.	4	0	2	84
54.	4	4	4	25

1/ Values outside confidence limits
2/ Total possible score 200 points

Section 11

REVISIONS TO THE QUALITY CONTROL MANUAL

This manual will be revised biennially and all persons on the mailing list will automatically be mailed copies of the revisions. The question then for each manual holder is whether his name is in fact on the list. Consider the following points:

1. If you received this manual or a set of revisions in response to a mail or phone request, you are definitely on the list.
2. If you received the manual as a handout at some training course, and your name and affiliation were not recorded, you are probably not on the list and, therefore, will not automatically receive revisions.
3. If you obtained your copy of the manual from some individual not associated with the Laboratory at Research Triangle Park, NC, you are probably not on the list and, therefore, will not automatically receive revisions.

. . . .

If, after reading the foregoing, there is a doubt that you may not be on the mailing list, please clip off the section below, complete it in full and mail it as shown to ensure that you will receive all future revisions.

TO: Quality Assurance Section, Anal. Chem. Br. (MD-69)
Environmental Toxicology Division
EPA, Health Effects Research Laboratory
Research Triangle Park, NC 27711

This is to request that your record be reviewed to be certain the undersigned is on your mailing list to receive copies of all future quality control manual revisions.

(Print or type name and full business address)